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Meta-Analysis of Wheat QTL Regions Associated with Heat and Drought Stress

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META-ANALYSIS OF WHEAT QTL REGIONS ASSOCIATED WITH HEAT AND DROUGHT STRESS

Meta-Analysis of Wheat QTL Regions Associated with Heat and Drought Stress

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Abstract

Heat and drought are the two most important environmental constraints to wheat production globally, are often present simultaneously and will become more severe with global climate change. This presents a unique challenge to wheat scientists who must work to develop wheat cultivars that are productive and adapted to future environmental conditions. A number of recent studies have reported quantitative trait loci (QTL) associated with heat and drought tolerance, as well as QTL for stress adaptive traits such as the availability of stem carbohydrates or crop canopy temperature. The objective of this study was to perform a meta-analysis of these QTL to identify regions of the wheat genome that are consistently associated with tolerance to heat and drought. To identify Meta-QTL (MQTL), a QTL database was developed from 30 studies targeted at heat and drought stressed environments. The positions of individual QTL were projected onto a consensus genetic map based on the presence of common molecular markers and a 95% confidence interval (CI) was calculated for each QTL. After positioning the individual QTL, the software 'Biomercator v2.1' was used to predict the location and CI of MQTL based on maximum likelihood.

In total, 854 QTL were reported for 80 different traits. This included 502 for drought stress, 234 for heat stress, and 118 adaptive trait QTL in non-stressed environments. These QTL were grouped into 66 MQTL regions distributed throughout the wheat genome. Most regions co-localized for both heat and drought stress, although both drought and heat stress specific MQTL regions were also identified. Using the traits present within MQTL it was possible to genetically model Stress Trait Expression Pathways (STEPs) that can be used to identify target alleles and physiological traits for improvement through breeding.

This thesis is approved for recommendation

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Dedication

To

Dr. James “Mac” Stewart,

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Chapter I
Introduction and Literature Review

Drought stress negatively impacts wheat yield and yield components

Drought stress is a primary constraint to wheat production, affecting as much as 42% of global production area with estimated yield losses as high as 50% (Kosina et al. 2007). In wheat, both total yield and yield components are greatly affected by drought conditions. Studies have shown that drought stress can reduce total wheat yield by 10 to 95% (Kilic and Yagbasanlar, 2010; Ozturk and Aydin, 2004; Reynolds et al. 1994; Foulkes et al. 2007; Sieling et al. 1994), with variation in yield loss depending on both the duration and phenological stage of the crop during stress. Ozturk and Aydin (2004) observed yield losses of 24, 41, and 66% in wheat that was drought stressed during mid-vegetative stage, mid grain-fill or for the entire season, respectively. A reduction of 48% for grain number per spike and 60% in spikes per unit area was observed in durum wheat under severe drought stress conditions (Giunta, 1993). In the same study, a decrease in harvest index of 24 to 48% was also observed. Villegas et al. (2001) reported a 40% decrease in final wheat biomass. Similarly, Lopes et al. (2011) reported reductions in yield of 61% as well as a 59% reduction in biomass. Foulkes et al. (2007) evaluated different physiological traits under drought stress and their association with grain yield losses and showed a clear correlation between high grain yield and leaf persistence, suggesting that this may be a trait for improvement through breeding.

The impact of drought stress on crop yield can vary greatly depending on the growth stage at the time of stress. Generally, the effect of drought stress during the vegetative stage can be overcome when the stress is present at an early stage of growth. Moreover, the recovery of the plant suggests a tolerance acquisition for future exposure to drought. Siddique and Hamid (2000) reported a reduction from 88 to 45% in relative water content during vegetative stage drought stress. However, measurements taken at the end of the vegetative stage showed a complete

recovery of the relative water content in the crop. Studies looking at drought stress at anthesis have reported varying results. For example, Sieling et al. (1994) reported that early anthesis is the most sensitive stage in terms of yield loss but this varied depending on the duration of stress. In contrast, Ji et al. (2010) found yield components such as kernel number and kernel weight to be greatly affected by reproductive stage drought stress, with losses in individual grain weight ranging from 30 to 40% and a reduction in grain number of 28% observed. Gooding (2003) reported a reduction in grain weight when drought stress was applied between the first fourteen days of anthesis and this reduction was associated with the premature ending of the grain filling process.

Heat stress negatively impacts wheat yield and yield components

Nearly 60% of the global wheat production area experiences some level of heat stress during the growing season (Kosina et al. 2007). Studies have shown that heat stress can reduce total wheat yield by 22 to 50% (Ferris 1998; Blum et al. 2001; Stone and Nicolas, 1994; Spiertz et al, 2006; Asseng et al. 2011). Ferris (1998) found that a four day heat stress treatment at anthesis significantly reduced yield production by 50%. Blum et al. (2001) reported a reduction of 46.8% in yield production and 43% in biomass of plants under heat stress during the entire life cycle. Stone and Nicolas (1994) reported a significant reduction in individual kernel weight, making it the trait most sensitive to high temperatures in their study. Asseng et al. (2011) observed a 50% reduction in yield production when ranges of optimum temperatures for wheat production vary by 2°C. Spiertz et al. (2006) proposed an increment in the tolerance to heat stress and showed that two applications of moderate heat stress during the vegetative stage could increase tolerance to high temperatures and reduce yield losses by 25% when severe heat stress is present during grain filling period.

Conceptual model of heat and drought stress adaptive traits

Yield under stress is highly variable and has been shown to be genetically different from yield under well-watered conditions (Messmer et al. 2009; Pinto et al. 2010). The use of target physiological, adaptive and morphological traits has become popularized as a means to higher yields under stressed conditions. This idea was first proposed by Passioura (1977) who developed a conceptual model that targeted better water use efficiency to improve harvest index and grain-yield under drought. This model has since been extended to include heat stressed environments and environments where heat and drought occur simultaneously (Reynolds et al. 2007). A summary of important heat and drought physiological adaptive traits that have been studied are presented in Table 1. These traits are a useful tool for selection of tolerant germplasm and identification of genotypes that can be used as a model for future studies. For example, canopy temperature is an important adaptive trait that allows for the identification and selection of tolerant cultivars even when stress is present in different phenological stages. A cooler crop canopy, or individual plant organ such as a flag leaf or main spike, serves as an indirect measurement of early growth which prevents evaporation of water, access to water by a deeper and more robust root system, and/or photo-protection by reflectance of excess heat. These traits can be important in both drought and heat conditions (Reynolds et al. 2005). In addition, canopy temperature has been correlated with high yielding, productive cultivars under both heat and drought conditions (Reynolds et al. 2007). Another example of an important adaptive trait is coleoptile vigor. The development of long coleoptiles allows for efficient uptake of water by the plant and facilitates the rapid establishment of the plant, therefore reducing soil evaporation and conserving moisture for the crop (Reynolds et al. 2005; 2007).

In order to understand and associate the physiological response of plants to conditions of drought and heat stress, a conceptual model was developed by Reynolds et al. (2005). The objective of this conceptual model was to identify the physiological traits associated with higher productivity and higher yield under stress and then target these traits for genetic improvement (Reynolds et al. 2007). The considered adaptive traits include; large seed size, long coleoptiles, early ground cover, high pre-anthesis biomass, good capacity for stem reserves and remobilization, high spike photosynthetic capacity, high relative leaf water content (RLWC) stomatal conductance (Gs), canopy temperature depression (CTD) during grain filling, osmotic adjustment, accumulation of abscisic acid (ABA), leaf anatomy (waxiness, pubescence, rolling, thickness, posture), and stay-green. These traits were then grouped into four principal categories; 1) Early growth (pre-anthesis growth), 2) access to water, 3) water use efficiency and 4) photo-protection. The association of these traits in different genetic backgrounds has made it possible to identify wheat genotypes that can be used as models for drought and heat adaptation and for genetic gain in yield (Reynolds et al. 2007). The goal of the present study is to use QTL meta-analysis to genetically explain key traits involved in the conceptual model of stress adaptation.

Physiological and biochemical effects of drought stress in wheat plants

Time and severity of drought conditions (Araus et al. 2002), phenological stage (Zhu et al. 2005), and genotype (Rampino et al. 2006) are factors determining plant adaptation to water stress. Fast and constant water stress can trigger a completely different mechanism than when the stress is slow and non-continuous.

In the first case, plants reduce water intake, and produce a constitutive or induced mechanism such as shoot growth inhibition, reduction in transpiration area, stress gene activation, metabolic acclimation, and osmotic adjustment in order to accumulate solutes such as

Na^+ (Mahajan and Tuteja, 2005) and sugars (Kameli and Losel, 1993). This accumulation increases water movement into the leaves for leaf turgor. These physiological changes lead to necrosis, growth development alteration and/or chlorosis in plant tissues (Mahajan and Tuteja, 2005).

In cases where the stress is gradual, escape or avoidance mechanisms are used by the plant (Barnabás et al. 2008). In addition, changes such as stomatal closure, carbon assimilation reduction, root signal recognition, growth inhibition, signal transport alteration, xylem hydraulic changes, and osmotic adjustment are triggered by the plant (Chaves et al. 2003).

Negative effects in plant generative organs under drought stress are pollen sterility, ovule abortion, reduction in sink potential (Barnabás et al. 2008), disruption in embryo cell division (Liu et al. 2005) and in the endosperm; leading to reduction in nutrient resources resulting in ovary abortion (Liu et al. 2005), and alteration in double fertilization process (Barnabás et al. 2008).

Genes involved in the drought stress response

At a molecular level, a variety of responses are triggered by the plant in order to avoid or reduce the negative effects of drought stress. For instance, water stress produces changes in chaperone synthesis (Mahajan and Tujea, 2005) and triggers the activation of different enzymes controlling reactive oxygen species (ROS) (Zhu 2002). Late embryogenesis abundant proteins (LEA) are accumulated under drought conditions (Rampino et al. 2006). These proteins help to prevent protein aggregation and plant metabolism alteration (Goya et al. 2005). ROS alteration impairs membrane lipids due to peroxidation and affects protein denaturation and DNA mutations (Bowler et al. 1992). Osmotic adjustment is one important response by the plant to water stress (Morgan, 1984). In Arabidopsis, the activation of AthTK1 under drought conditions

is triggered by osmotic stress in the plant. The AthTK1 is a histidine kinase with osmosensor capacity, being the stress signal for the mitogen activated kinases (MAPK) cascade activation (Urao et al. 1999).

An increase in abscisic acid (ABA) produced by reduction in leaf and root turgor (Loss and Siddique, 1994) is one of the principal responses of the plant at any growth stage to adverse water conditions (Davies and Zhang, 1991; Barnabás et al. 2008). ABA production leads to stomatal closure, alteration in the transpiration process (Loss and Siddique, 1994), increment in root hydraulic conductivity (Barnabás et al. 2008), and reduction of leaf growth during vegetative stage as a mechanism to reduce water loss through transpiration inducing stomatal closure (Liu et al. 2005). Moreover, stomatal closure is followed by Rubisco inactivation resulting in the reduction of the photosynthetic process (Bota et al. 2004).

A number of secondary metabolites have been shown to be synthesized under drought stress. Osmoprotectant' oligosaccharides such as raffinose, galactinol (Taji et al. 2002), amino acids such as proline, glutamate and sugars such as mannitol and sorbitol are produced, providing cell membrane protection (Mahajan and Tuteja, 2005). However, the specific role of proline is still debatable. Rampino et al. (2006) reported a reduction in relative water content with a significant increase in proline in sensitive wheat genotypes under drought conditions, suggesting an adverse effect under water stress.

Physiological and biochemical effects of heat stress in wheat plants

Under conditions of heat stress, important processes such as enzymatic function, protein synthesis, membrane thermostability and photosynthesis can be compromised (Berry and Bjorkman, 1980), resulting in cellular death and high levels of oxidative stress (Kotak et al. 2007). In order to tolerate high temperature stress, plants have developed physiological and

biochemical adaptive strategies that maintain photosynthetic rate, activate heat shock proteins and increase respiratory rate (Mason et al. 2011). Respiration is linked to plant growth, growth maintenance, and protein synthesis (Gifford, 2003). Under heat conditions, Almeselmani et al. (2012) reported a respiration rate adaptation in wheat plants, which allows growth development and tolerance to high temperatures. The initial physiological response of wheat to heat stress is an increased rate of plant development resulting in a general reduction in plant size at maturity (Ayeneh et al. 2002). Heat stress decreases both yield and quality of the grain, principally due to its negative effect on photosynthesis and translocation of carbohydrates (Wang et al. 2011). Xu et al. (2001) reported a reduction of photosynthesis after 10 days of continuous high temperatures due to chloroplast disruption, where 47.3% of cells were broken as a result of heat stress. Moreover, high temperature affects pollen production, pollen tube germination, the fertilization process, and ultimately results in sterility in wheat plant reproductive systems (Ferris 1998).

An acclimation response to high temperatures is produced by plants. In fact, an increment in heat tolerance was reported when wheat plants were subjected to short periods of high temperatures before anthesis (Wang et al. 2011). Moreover, an increment in the cell membrane thermostability in the peduncle, sheath, glume, and awns was reported by Xu et al. (2001). This acclimation can be inherited by the progeny, and allows a fast response by the plants under future adverse heat conditions (Wang et al. 2011).

During the grain filling period, high temperatures inhibit production and transport of photoassimilates resulting in starch synthesis inhibition and reduction in yield and grain quality (Bahar and Yildirim, 2010). Evapotranspiration also plays an important role under heat stress, reducing leaf and spike temperature (Ayeneh et al. 2002). Quality and dough are affected by high

temperatures by the glutenin polymerization of subunits during the grain filling period (Irmak et al. 2008). Individual kernel weight is affected during heat stress present before anthesis by endosperm, composition, and maturating embryo disruption (Mason, 2011).

Genes involved in the heat stress response

High temperatures produce a complex response in plants at the cellular and molecular level. This involves an increase in the concentration of salicylic acid, ethylene, an increase in saturated and monosaturated fatty acids and increased expression of heat shock proteins. (Hays et al. 2007; Penfield, 2008). HSPs are chaperones that provide a partial thermo resistance for the plant (Schoffl et al.1998; Priti, 2003; Kotak et al. 2007). Different types of HSPs have been reported in several organisms (Schoffl et al. 1998). Abscisic acid and brassinosteroid production have been linked with the expression of HSP and the development of thermotolerance (Priti, 2003). Skylas et al. (2009) identified seven heat shock proteins of low molecular weight which were expressed in the heat tolerant wheat cultivar 'Fang'. HSPs are classified in five families based on molecular weight: HSP60, HSP70, HSP90, HSP 100 and small HSP (Wang et al. 2004). The synthesis of HSPs begins when temperatures rise above 32° C (Vierling, 1991). HSPs are key factors in membrane protein and receptor activity regulation and in oligomeric protein production and protein folding (Vierling, 1991). For some HSPs, specific functions have been determined. For example, HSP70 is associated with protein structure definition and ATP protein folding (Vierling, 1991). HSP 60 has a key role in protein maturation in the mitochondria (Welch, 1993) and is implicated in CO2 fixation in the chloroplast with the participation of rubisco (Ellis, 1990). Rubisco activase allows the carbamylation of rubisco, a process that is indirectly affected by high temperatures in which heat produces an accumulation in Rubisco activase which slows down photosynthesis and rubisco deactivation (Ristic et al. 2009). The

HSP60 and HSP70 interact during the protein maturation process, so it is believe that these two families work together in the maturation process (Welch, 1993). The HSP90 family has several roles, including the maintenance of signal transduction between proteins, protein degradation, and morphological evolution, in which cell structure can be modified by HSP90 in order to avoid mutations or generated adaptations to specific environmental condition at cellular level (Young et al. 2001). The main function of the HSP100 family is to remove proteins that can disturb cellular homeostasis and which are produced by incorrect processes in the protein folding (Wang et al. 2004). Heat shock factors (HSFs) are the transcriptional regulators of HSPs. HSFs are composed of four families, HSF1, HSF2, HSF3 and HSF4. Three of the four families (HSF1, HSF2, and HSF4) have active cross talk and are involved in controlling a variety of physiological processes. HSF1 is recognized as the main regulator in the HSP activation response (Akerfelt et al. 2010)

Wheat genetics and genetic resources

Common bread wheat is an allohexaploid, with its genome evolving from three different species (Mukai et al. 1993, Chenuil et al. 1999). Each of the three wheat genomes is composed of seven chromosomes (1-7), resulting in three homeologous chromosomes (A, B and D) across the three genomes (Gill et al. 2004). The hexaploid genome of *Triticum aestivum* contains the A, B and D genomes, which originated from three related species (Mukai et al. 1993); *T. monococcum* (A), a relative of *Aegilops speltoides* (B) and *Aegilops tauschii* (D). Sympatry between the genomes of emmer (AABB) and *Aegilops tauschii* (genomes DD) ultimately resulted in hexaploid wheat (Dubcovsky and Dvorak, 2007). The estimated genome size for hexaploid wheat is 15,966 and 13,000 Mbp for tetraploid durum wheat (*Triticum turgidum*) (Arumuganathan and Earle, 1991). The genome is approximately five times the size of the

human genome and 45 times larger than the sequenced genome of rice, making it one of the largest and most complex crop genomes (Argumuganathan and Earle, 1991). Due to the sheer size as well as the highly repetitive DNA structure there is currently no complete annotated genome sequence of wheat. However, considerable effort has been put toward developing a complete wheat genome sequence, and the annotated sequence of chromosome 3B is near completion (Paux et al. 2008)

Molecular markers are "tags" related with key agronomic and physiological traits in chromosomal regions of an organism (Collard et al. 2005). Many different classes of molecular markers are used in breeding and genetic studies. Two of the most common markers used today include simple sequence repeats (SSRs) and more recently single nucleotide polymorphisms (SNPs) (Gut, 2001). SSRs are markers which identify a tandem of sequence repetitions in an organism's genome. These repetitions can be mononucleotide, or groups of di or trinucleotide repetitions (Ellegren, 2004). In wheat, SSRs are abundantly available and provide a medium-throughput option for molecular marker analysis (Somers et al. 2004). On the other hand, SNPs are changes in a unique, single nucleotide in a gene sequence. A recent genetic map integrating 1,114 SNPs markers demonstrate their utility in wheat research (Allen et al. 2011). However, due to the low rate of polymorphism in SNP markers, SSRs have been the most suitable markers for genetic studies in wheat to date (Gana and Röder, 2007).

Quantitative trait loci regulating heat stress and drought stress tolerance

In genetics, molecular markers are frequently used in mapping studies to identify quantitative trait loci (QTL) within a genome. A QTL is a genomic region statistically associated with a trait of interest. This statistical association involves the use of linear and multiple regression to associate differences in a molecular marker allele with differences in a trait of

interest. This statistical analysis is carried out using molecular mapping software such as QTL Cartographer (Wang et al. 2007). The basic steps involved in a QTL mapping study are outlined in Figure 1. In addition to the genome location, the variance explained (R^2), additive genetic effect, significance (LOD) and favorable parental allele are also gathered from a QTL analysis. This data can then be used to determine the overall genetic control of a trait and which important QTL might be used for a marker-assisted breeding approach.

Despite the importance of heat and drought stress, our understanding of their genetic control in wheat, such as the genes and QTL regulating these traits, is still very limited. However, a number of recent studies have used QTL mapping to identify yield, agronomic and physiological traits that are associated with improved heat and drought tolerance. The first wheat study mapping a genomic region associated with heat tolerance was carried out by Yang et al. (2002). In this study, two molecular markers, one each on chromosomes 1B and 5A were found to be associated with grain-filling duration under reproductive stage heat stress. In subsequent studies, a susceptibility index has often been used as a measurement of yield reduction under stressed versus non-stressed conditions (Fischer and Maurer, 1978). An association between heat susceptibility index (HSI) and 27 heat tolerance QTL was reported by Mason et al. (2010) under a short period of heat stress in early grain filling. A follow up study by Mason et al. (2011) identified 14 QTL associated with maintenance of the yield components kernel number, individual kernel weight and total kernel weight, and confirmed 7 genomic regions from the Mason et al. (2010) study. In this study, the individual QTL explained between 4.5 and 19.3% of the phenotypic variance for the traits. A similar study by Mohammadi et al. (2008) identified three QTLs associated with HSI in which 16.7% of the total phenotypic variation was explained. Vijayalakshmi et al. (2010) used measurements of chlorophyll content and chlorophyll

fluorescence in order to identify 14 QTL associated with wheat senescence cultivars under heat conditions.

Currently, a large amount of effort is being put toward the study and identification of QTL associated with drought tolerance. Golabadi et al. (2011) used four different irrigation conditions to identify QTL for yield and yield components and reported six specific drought QTL on chromosomes 2B, 3B, 6A and 7B in a durum wheat population. These QTL explained from 11.8 to 26.5 % of the phenotypic variance for traits such as harvest index, thousand kernel weight and grain number per spike. Kirigwi et al. (2007) identified an important region on chromosome 4A under drought conditions associated with grain yield, grain fill rate, spike density, grains m^{-2}, biomass production, biomass production rate, and drought susceptibility index (DSI). Peleg et al. (2009) evaluated 153 recombinant inbred lines (RILs) under three different moisture conditions, identifying 20 specific drought QTL associate with traits such as grain yield, spike dry matter, total dry matter, harvest index, carbon isotope ratio, osmotic potential, chlorophyll content, flag leaf rolling, culm length, days from planting to heading, and days from heading to maturity. Pinto et al. (2010) imposed both heat and drought conditions on a wheat recombinant inbred line population derived from two historically important wheat varieties ('SeriM82' and 'Babax') developed at the International Wheat and Maize Improvement Center (CIMMYT) in Mexico and reported 17 QTL for heat and drought tolerance in association with agronomic and physiological traits. These traits included grain number, thousand grain weight, anthesis, maturity, canopy temperature, vegetative index in the grain fill stage, and chlorophyll in the grain fill stage. Other studies have also targeted multiple stresses, including drought and salt stress (Quarrie et al. 2005) and drought, heat, and nitrogen stress (Zheng et al. 2010). In the study by Zheng et al. (2010), three individual QTLs were identified for both heat

and drought tolerance in association with kernel number, with the phenotypic variation explained ranging from 4 to 34%.

While these studies provide insight into the genetic control of heat and drought tolerance, their complex and quantitative genetic control continues to make improvement of these traits through molecular breeding difficult, with most of the current progress continuing to be made through phenotypic selection (Fleury et al. 2010). The information that is lacking is the validity of these QTL regions in additional genetic backgrounds and in different environments, which is often unknown given the specific target regions and populations of most studies. Therefore, the goal of this proposal is to use a meta-analysis approach that allows for the combined analysis of data across studies and for the identification of consistent, stable QTL regions.

Previous studies using meta-QTL analysis in plants

A meta-analysis is a statistical technique used to analyze data from different studies on the same topic (Goffinet and Gerber, 2000). Meta-analysis has been used to study a number of different agronomically important traits in various crop species. In wheat, meta-analysis has been applied to study Fusarium head blight resistance (Liu et al. 2009; Löffler et al. 2009), identification of quantitative traits such as grain protein content, preharvest sprouting tolerance, grain weight (Gupta et al. 2007), seed dormancy (Tyagi and Gupta, 2012), earliness traits (Hanocq et al. 2007), and QTL related to yield and yield components (Zhang et al. 2010).

In other crop species, meta-analysis has been used to study height (Sun et al. 2012) and cyst nematode resistance QTL (Guo et al. 2006) in soybean, disease resistance in cacao (Lanaud et al. 2009), blight resistance and plant maturity traits in potato (Danan et al. 2011), fiber quality in cotton (Lacape et al. 2010) and traits associated to drought, cold temperatures, waterlogging,

salt content and mineral availability in barley (Wei et al. 2012). Swamy et al. (2011) showed that meta-QTL associated with yield under drought stress in rice were conserved across grass species.

Approach of the current study

While recent studies provide insight into the genetic control of heat and drought tolerance, our knowledge is still lacking, as often times the validity of QTL regions detected in a single study are not applicable across environments and genetic backgrounds. The *objective of the current study* was to identify regions of the wheat genome which are consistently associated with heat and drought stress tolerance. The *central hypothesis* is that the most significant QTL controlling heat and drought stress tolerance can be detected across genetic populations using a meta-analysis. The following *specific objectives* were used to test this hypothesis:

Objective 1: Conduct a meta-analysis of wheat QTL regions associated with heat and drought tolerance in order to identify meta-QTL (MQTL). This objective was accomplished by analyzing the results of 30 QTL mapping studies targeted at heat and drought stress or related physiological traits. The working hypothesis is that important QTL expressed across studies and in different genetic backgrounds will be detected as MQTL.

Objective 2: Determine the allelic diversity of MQTL in a panel of 74 soft red winter wheat lines from the Southeastern U.S. breeding programs. Simple sequence repeat (SSR) markers linked to the most significant MQTL regions identified in Objective 1 were used for marker analysis. The working hypothesis is that significant genetic variation exists within the soft red winter wheat germplasm for heat and drought tolerance.

The MQTL regions identified, and the study of the allelic diversity in the 74 wheat lines will allow for the future development of single nucleotide polymorphism (SNP) markers for fine mapping, the identification of candidate genes and markers for marker assistant breeding.

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Table 1. Important physiological adaptive traits in wheat

Trait category	Method of Measurement	Importance
Biomass	Sampling of plant tissue at different stages of development	A component of water-use efficiency, highly correlated with yield
Canopy reflectance	Measured using a spectral radiometer	Evaluation of the total photosynthetic area, reflected light and absorbed light
Canopy temperature	Measured with a hand-held thermometer	Surrogate for stomatal conductance, water uptake, heat reflectance.
Carbon isotope discrimination	Measure difference between the isotopic ratio of ^{12}C and ^{13}C	Surrogate trait for transpiration efficiency
Chlorophyll or stay-green	From 400nm to 700nm measured with a chlorophyll meter	Component of photosynthesis and prolonged grain filling
Coleoptile vigor	Measure length of coleoptile at the seedling stage	Improves plant establishment and rapid emergence
Cuticular wax	Visual score of abxial flag leaf wax	Decreases radiation to the leaf surface and evapotranspiration rate
Grain-filling duration	Duration is days from anthesis to senescence	Determinant trait in yield,sensitive to drought and heat
Photosynthesis	Quantum yield and chlorophyll fluorescence using a fluorometer	Photosynthesis produces carbohydrates for grain-fill
Root biomass and vigor	Washing and/or examining of roots under varying conditions	Large and vigorous root systems have more access to water
Water soluble carbohydrates	Measure by chromatography or near infrared spectroscopy.	Carbohydrate reserves can be allocated to the grains under stress
Water status	Samples of fresh weight and dry weight or pressure bomb	Determines water absorption, osmotic potential and water use efficiency
Yield components	Sampling of spike bearing culms at maturity for yield components	Many yield components are correlated with total yield
Total yield	The total grain weight of experimental plant or plot	Most economically important trait

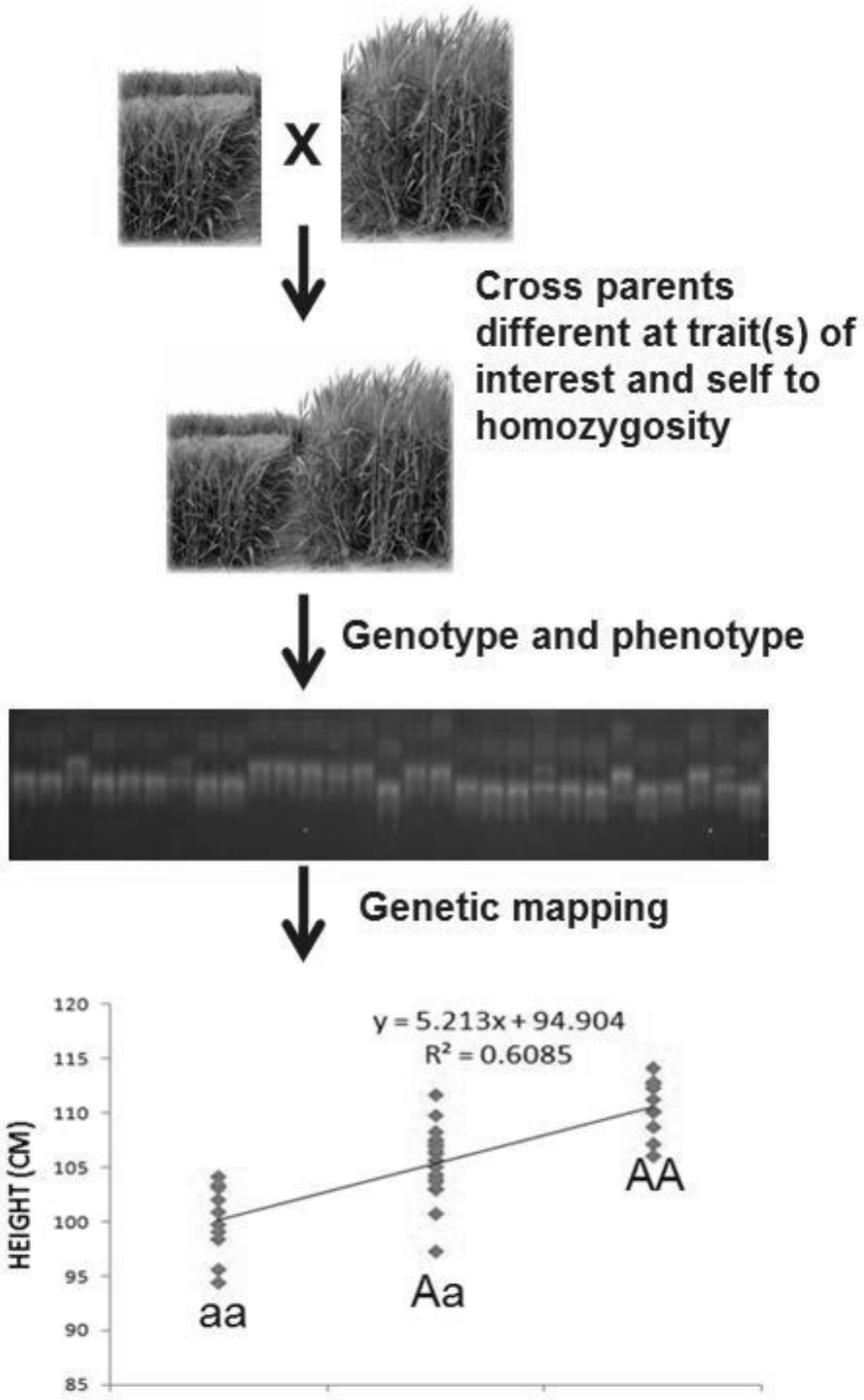

Fig. 1. Basic steps involved in a QTL mapping study

Chapter II
Meta-Analysis and Allelic Diversity Study

Abstract

The goal of this study was to identify stable quantitative trait loci (QTL) associated with heat and drought tolerance in wheat using a QTL meta-analysis approach. To identify Meta-QTL (MQTL), a QTL database was developed from 30 studies targeted at heat and drought stressed environments. The positions of individual QTL were projected onto a consensus genetic map based on the presence of common molecular markers. A 95% confidence interval (CI) was then calculated for each QTL. After positioning the individual QTL on the consensus map, the software 'Biomercator v2.1' was used to predict the location and CI of MQTL based on maximum likelihood.

In total, 854 individual QTL were reported for 80 different traits. This included 502 for drought stress, 234 for heat stress, and 118 adaptive traits QTL in non-stressed environments. Meta-analysis identified 66 MQTL regions distributed unevenly throughout the wheat genome, but with at least one MQTL detected on each of the 21 wheat chromosomes. There were 43 MQTL regions which co-localized for both heat and drought stress, twenty specific for drought stress, and two specific for heat stress. A reduction in the average 95% CI from 21.6 cM to 5.8 cM was observed when comparing the CI of individual QTL present within MQTL regions to the CI of the MQTL, respectively.

The majority of MQTL identified were in agreement with previous MQTL studies in wheat, including regions previously detected for yield, yield components and major genes such as *Ppd-D1* and the *Rht* genes controlling plant height. However, this study provides new insight into these QTL regions by identifying additional agronomic, physiological and adaptive trait QTL present in these regions that collectively contribute to improved yield potential and stress tolerance. Using the traits present within MQTL and physiological models describing the role of

adaptive traits in abiotic stress tolerance, it was possible to genetically model Stress Trait Expression Pathways (STEPs) that can be used to identify target alleles and physiological traits for improvement through breeding.

Introduction

Heat and drought stress are the two most important environmental constraints to wheat production globally and will become more severe as a result of climate variability. Both of these abiotic stresses lead to complex biochemical and physiological changes in the plant, resulting in accelerated growth, lower biomass, premature senescence and ultimately, lower grain yield. Due to the complexity of the plant's response to abiotic stress, an integrated approach that combines molecular genetics and genomics with precision phenotyping of important adaptive traits is utilized. This integrated approach is vital if breeders are to identify new sources of genetic variation and incorporate these novel trait alleles into wheat varieties that are adapted to future environmental conditions.

The use of target traits in wheat as a means to higher yield in stressed environments was first proposed by Passioura (1977) who developed a conceptual model that targeted better water use efficiency to improve harvest index and grain-yield under drought. This model has since been extended to include an array of physiological traits that are associated with higher productivity in both drought and heat stressed environments and environments where heat and drought occur simultaneously (Reynolds et al., 2007). The objective of this conceptual model is to identify the traits most highly correlated with higher plant productivity and higher yield in a target environment and then target these traits for genetic improvement through breeding (Reynolds et al. 2007). Examples of adaptive traits include; large seed size, long coleoptiles for early ground cover, high pre-anthesis biomass, good capacity for stem carbohydrate reserves and

remobilization, high spike photosynthetic capacity, high relative leaf water content, stomatal conductance, canopy temperature measurements, osmotic adjustment, accumulation of abscisic acid, leaf anatomy such as posture or waxiness, and stay-green. Grouping of these traits into four principal categories; 1) pre-anthesis growth, 2) access to water, 3) water use efficiency and 4) photo-protection, allows for trait selection depending on the target environment and at what point of development stress is likely to occur. While 'physiological breeding' is still in its infancy, at least in comparison to traditional phenotypic selection for qualitative traits and yield, some successes have been reported. Early generation selection for low carbon-isotope discrimination as a surrogate for high transpiration efficiency was successful in developing wheat genotypes with higher above ground biomass, harvest index and grain yield in low rainfall environments (Rebetzke et al., 2002). Likewise, Reynolds et al. 2007 showed that theoretical yield increases for adaptive trait selection ranged from 3 to 14% under drought stress and from 2 to 24% under hot, irrigated conditions when comparing the highest expressing to lowest expressing lines for a given adaptive trait.

While phenotypic selection for physiological traits is less complex than selecting for yield *per se*, many of these target traits remain cumbersome and expensive to measure and are lowly heritable across environments. For example, carbon isotope discrimination requires a large amount of tissue sampling, in many environments, and analysis with mass spectrometry, which makes it amenable to a small scale experiment but less so to a large scale breeding program. An alternative to phenotypic selection is the development of marker-trait associations via quantitative trait loci (QTL) mapping and subsequent selection of traits based on the presence of molecular marker alleles. QTL mapping has been applied to detect genome regions associated with a number of traits in nearly every major crop species, including under abiotic stress

(Tuberosa et al., 2002). However, only recently has an emphasis been put toward identifying QTL associated with physiological traits and their relationship with heat and drought tolerance.

Yang et al, (2002) identified the first genomic region associated with heat tolerance in wheat, identifying two markers associated with grain-filling duration under continuous heat stress during reproductive development. The use of a susceptibility index is often employed to identify lines that show minimal reduction in yield or yield components in stressed versus non-stressed conditions (Fischer and Maurer, 1978). QTL associated with heat susceptibility index (HSI) have been reported (Mohammadi et al. 2008, Mason et al. 2010; 2011), and QTL for lower HSI have been shown to co-localize with QTL for lower leaf and spike temperature (Mason et al. 2011). Other heat stress QTL studies have focused on various adaptive and agronomic traits, including leaf senescence (Vijayalakshmi et al. 2010), canopy temperature, chlorophyll content and normalized differential vegetative index (Pinto et al. 2010) or by analyzing QTL x environmental effects across multi-environmental trials (Kuchel et al.2007; Zheng et al. 2010).

Given its importance in so many wheat growing environments, a greater focus has been put toward identifying QTL associated with drought stress tolerance and the related adaptive traits. This includes studies focused on yield and yield components (Golabadi et al. 2011, Quarrie et al. 2005), carbon isotope discrimination (Maccaferri et al. 2008; Peleg et al. 2009; Rebetzke et al. 2008), canopy temperature (Diab et al. 2008; Pinto et al. 2010) carbohydrate reserves (Snape et al. 2007; Teulat et al. 2001; Yang et al. 2007a), and drought susceptibility index (Kirigwi et al. 2007; Peleg et al. 2009).

While the traditional bi-parental QTL mapping approach is powerful, it has limitations. In many cases, the significant QTL identified in any single study may not be applicable or are simply not tested in different genetic backgrounds and different environments. The meta-analysis

approach was developed to combine results from individual QTL studies into a single dataset and identify the most likely position and confidence interval of QTL regions (Goffinet and Gerber, 2000). Meta-analysis combines the statistical power of multiple QTL studies, generally resulting in a smaller QTL confidence interval compared to individual studies. Meta-analysis has been employed in a number of different crop species for a range of traits. Examples from other crops include drought tolerance in rice (Swamy et al. 2011), height (Sun et al. 2012), soybean cyst nematode (Guo et al. 2006), disease resistance in cacao (Lanaud et al. 2009), blight resistance and plant maturity traits in potato (Danan et al. 2011), tolerance to abiotic stresses in barley (Li et al. 2012) and fiber quality in cotton (Lacape et al. 2010). In wheat, meta-analysis has been used to identify meta-QTL for *Fusarium* head blight resistance (Liu et al. 2009, Löffler et al. 2009), quantitative traits such as grain protein content, preharvest sprouting tolerance, grain weight (Gupta et al. 2007), preharvest sprouting and dormancy (Tyagi and Gupta, 2012), earliness (Hanocq et al. 2007), and yield and yield components (Zhang et al. 2010).

In most QTL studies, multiple traits are measured in order to understand the pleiotropy underlying a trait of interest. Meta-analysis has the ability to combine QTL from many studies for any number of traits and allows for the dissection of a complex trait, such as yield, into its component traits beyond what is possible in any single QTL study. In this study, we developed a QTL database from 30 different studies targeted at heat and drought stressed environments or at adaptive trait identification in non-stressed environments. These QTL were placed onto a consensus genetic map and MQTL were identified. Finally, we integrated results from the MQTL analysis with conceptual models for stress adaptation to hypothesize examples of Stress Trait Expression Pathways (STEPs) that could genetically explain, based on the MQTL results within a genome region, the physiological response of wheat to heat and drought stress.

Materials and methods

Database development

A database containing the QTL profiles of 30 studies targeted specifically at heat and drought tolerance and/or adaptive physiological traits was developed (Table 1). This included 25 hexaploid (ABD) and five tetraploid (AB) wheat studies. For each QTL, information was collected on the chromosomal location, most closely linked marker, favorable allele, LOD value, and R^2 value.

Projection of the individual QTL on a consensus genetic map

After the development of the QTL database, all individual QTL were projected onto a consensus genetic map developed by Somers et al. (2004). This map contains 1,235 marker loci, making it one of the most saturated SSR genetic maps available. Nearly every published genetic map in wheat shares common markers with the consensus map, making it suitable as a reference map for a meta-analysis. Individual QTL were projected onto the consensus map using the consensus position of common SSR markers. In cases where the individual genetic map and the consensus map share a common SSR marker, the QTL positions were projected based on the cM position of the common SSR in the consensus map. In cases where a common marker was not available between the individual genetic map and the SSR consensus map, a third genetic map was used as a cross reference. The cross referenced genetic maps used in this study included the CIMMYT integrated map (Crossa et al. 2007), Seri/Babax (McIntyre et al. 2010), Synthetic/Opata (Roder et al. 1998), Nanda 2419/Wangshuibai (Xue et al. 2008), and the *T. turgidum*, Langdon/G18-16 map (Peleg et al. 2008). Where a suitable marker could not be identified (> 5cM) QTL were eliminated from further analysis.

Calculation of 95% confidence intervals for individual QTL

In a genetic mapping study, the confidence intervals of QTL are generally presented as one or two-LOD intervals. However, these confidence intervals are biased by the population size, marker density of the genetic maps and type of population used in an individual study. For a meta-analysis, it is necessary to assess the CI using the same method for all studies. Therefore, the following formulas were used to determine a 95% confidence interval for each of the individual QTL:

$CI= 163/(NR^2)$ *For Recombinant Inbreed Lines* (Guo et al. 2006)

$CI = 287/(NR^2)$ *For Double Haploid Lines* (Liu et al. 2009)

where N is the population size and R^2 is the variation explained by the QTL

The confidence interval of an individual QTL is therefore dependent on the factors that increase the statistical power and accuracy of a QTL study, including the population size (N), the variation explained by the QTL (R^2) and the amount of meaningful recombination. The recombination factor is dependent on the type of population used in the study, which is 163 for recombinant inbred lines and 287 for double haploid lines.

Meta-analysis using Biomercator

Biomercator v2.1 was used to carry out the QTL meta-analysis. This software is based on the algorithm developed by Goffinet and Gerber (2000) in which a maximum likelihood function is produced in order to determine the number of MQTL given a set of input QTL on a common genetic map, a consensus position of each MQTL based on the variance of QTL positions, and finally, a 95% CI for each MQTL based on the variance of input QTL intervals (Arcade et al. 2004). A number of previous meta-QTL studies have utilized this software (Zhang et al. 2010,

(Khowaja et al. 2009). Only those MQTL with individual QTL from ≥ 2 studies were considered to be Meta-QTL.

Plant materials and DNA isolation for allelic diversity analysis

The most significant twenty MQTL regions were used for marker analysis. A panel of 74 lines from the Gulf Atlantic Wheat Nursery (GAWN) (Table 2) containing advanced breeding lines from Arkansas, Florida, Georgia, Louisiana, North Carolina, South Carolina, and Virginia were used for genotyping. Seedlings of each line were germinated on filter paper in petri dishes. A total of 100µg of fresh tissue was harvested from five seedlings per line and bulked into 1.5ml microtubes. Tissue was ground using steel beads in a TissueLyser II from Qiagen. Following grinding, DNA was extracted using a wheat and barley protocol as described by Pallotta et al. (2000).

Microsatellite markers, polymerase chain reaction and fragment analysis

A set of 21 wheat SSR primers linked to MQTL (Table 3) were used for allelic diversity analysis. Markers were assayed in 10µl PCR reactions containing; 10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM MgCl2, 0.2mM dNTP, 25ng of each primer, 20ng genomic DNA and 0.5U Taq polymerase. Conditions for PCR were as follows: 94°C for 3min, 35 cycles of 94°C for 1min, 50/55/60/61/°C for 50sec, and 72°C for min, followed by 5min at 72°C. SSR markers were screened for polymorphisms using the AdvanCE FS96 capillary electrophoresis (CE) system. This system allows DNA detection as low as 5pg/µL with a 2 bp resolution. For CE analysis, samples were prepared with 4µl of PCR reaction plus 44µl of TE (1x) buffer. Prosize software was used to determine allele sizes. For the diversity analysis, Powermarker software was used (Liu and Muse, 2005).

Results

QTL Database

Results from 30 studies were used to develop the QTL database. In total, 854 individual QTL were reported and characterized. The number of QTL reported in the individual studies ranged from 2 to 206, while the population size ranged from 34 to 249 lines. Twenty four different populations were evaluated in the 30 studies, including thirteen recombinant inbred lines (RIL) and eleven double haploid (DH) populations. Moreover, twenty five hexaploid populations (ABD) and five tetraploid populations (AB) were evaluated in the 30 studies.

From the 30 studies, QTL were reported for 87 different traits (Table 4) and these traits could be grouped into 21 general categories. Of the 854 reported QTL, 502 QTL were associated with drought tolerance, 234 with heat tolerance, and 118 were associated with adaptive traits in non-stressed environments. Of the drought stress QTL, 255 (51%) were associated with a physiological trait. Of the heat stress QTL, 137 (59%) were associated with a physiological trait (Fig. 1). QTL reported were evenly distributed between the A and B genomes when tetraploid studies were excluded from analysis, with 191 and 188 QTL, respectively (Fig. 2). However, the D genome had significantly fewer QTL, with 147 reported (Fig. 2). Overall, QTL were unevenly distributed across the 21 wheat chromosomes, ranging from as few as 11 on chromosome 3D to as many as 72 on chromosome 2B (Fig. 3).

Figure 4 and Figure 5 show the distribution for categories of agronomic and physiological trait QTL, respectively, across the 21 wheat chromosomes. For agronomic QTL, the traits thousand kernel weight (18%), biomass (14%), kernel number (14%) and yield (11%) were the most frequently reported (Fig. 4). For physiological traits, QTL related to

photosynthesis (21%), water status (17%), stay-green (16%) and carbon isotope discrimination (14%) were the most frequently reported (Fig. 5).

MQTL Analysis

From the initial pool of 854 individual QTL, 534 QTL were successfully projected onto the consensus map and used in the meta-analysis. The remaining 320 QTL lacked a tightly linked consensus marker mainly due to a lack of SSR markers present in the published genetic maps developed in these studies. A total of 66 MQTL were detected using Biomercator v2.1 (Table 5, Fig. 6) with at least one MQTL detected on each of the 21 wheat chromosomes. There were 43 MQTL regions which co-localized for both heat and drought stress, twenty specific for drought stress and two specific for heat stress. The number of individual QTL per MQTL ranged from 2 to 42 while the number of traits per MQTL ranged from two to 19. Likewise, the number of studies represented by each MQTL ranged from two to thirteen. A reduction in the average 95% CI from 21.6 cM to 5.8 cM was observed when comparing the individual QTL to the MQTL.

Major QTL clusters (≥ 15 individual QTL) were identified in 8 MQTL regions, including clusters on 1B, 2B, 2D, 4A, 4B, 4D, 5A and 7A. Many of these large clusters can be explained by the presence of major genes including MQTL on 1B (1B:1R translocation), 2B (*Ppd-B1*), 2D (*Ppd-D1*), 4B (*Rht-B1*) and 4D (*Rht-D1*) (Blake et al. 2009). Chromosome 5A is known to carry at least two major genes influencing yield, *Vrn-A1* and *q* genes regulating shattering, although the major QTL cluster at MQTL39 on 5AS appears to be located distally to the location of these genes. Twenty-eight MQTL regions showed moderate clustering ($15 > n > 5$). Thirty of the MQTL regions detected contained five or fewer individual QTL, with the number of studies represented by these regions ranging from two to four and the number of traits ranging from two to five.

Allelic diversity study

Major frequency allele, number of alleles, allele diversity, and polymorphism information content (PIC) values were calculated for SSR markers linked to 20 significant MQTL using PowerMarker (Liu and Muse, 2005). Allelic variation of the MQTL selected for evaluation and the SSR markers linked to the MQTL are presented in the Table 6. Twenty one SSR markers were used to determine gene diversity of 74 wheat lines from the Gulf Atlantic Wheat Nursery. A total of 346 alleles were detected. The allele number obtained in this study was in agreement with Zhang et al. (2002) in which they suggested a range between 350 and 400 alleles for allelic diversity studies and development of stable phylogenetic trees in wheat. The number of alleles per locus ranged from seven for *gwm388* to 24 for *gwm484* with an average number of 16.5 alleles per locus (Table 6).

Allele diversity for the 21 SSR markers evaluated ranged from 0.65 to 0.93. The largest allelic diversity was observed for markers *gwm484* and *gwm156* on chromosomes 2D and 5A with polymorphic information content (PIC) of 0.93, while the marker *gwm388* on chromosome 2B presented the lowest PIC value with 0.61. These results suggest that chromosomes 2D and 5A have a high level of genetic variation for these loci.

Moreover, the average PIC for the 21 markers was 0.83 (Table 6). A high correlation coefficient was observed between gene diversity and number of alleles with r = 0.7892 (Fig. 7). The markers were evaluated for polymorphisms using the AdvanCE FS96 capillary electrophoresis (CE) system, which was able to detect differences as small as 2 bp between fragments. For example, the marker *gwm156* shows molecular sizes between 283 and 316 bp (Fig. 8).

Discussion

Yield is a complex trait, involving a large number of genes with small effects and interactions between genes in the form of epistasis, ultimately resulting in expression of a phenotype. Yield under stress is highly variable and has been shown to be genetically different from yield under well-watered conditions (Messmer et al., 2009; Pinto et al., 2010). The use of physiology in combination with QTL mapping provides a powerful approach and has recently been utilized to identify several individual heat and drought stress QTL. However, even more statistical power and insight can be gained by combining individual studies into a single meta-analysis. From 30 studies focused on heat and drought stress tolerance, 854 individual QTL were identified for 81 important physiological and agronomic traits. These QTL were grouped in 66 MQTL regions which were associated with two to 19 different traits per region. Several MQTL identified in this study were in agreement with genomic regions previously identified (Zhang et al. 2010). Fourteen MQTL regions were in agreement with Zhang et al. (2010) in which genomic regions associated with yield and yield components were identified. This includes regions on chromosomes 1A, 1B, 2A, 3B, 4A, 4B, 4D, 5A. However, novel QTL regions on chromosomes 1A, 3B, 3D, 6B, and 7B were also identified. A similar MQTL study conducted in barley identified 79 MQTL, including regions for drought, low temperature, salinity, waterlogging and mineral deficiency and toxicity (Li et al. 2012). This included a number of physiological traits, for example soluble carbohydrates, carbon isotope discrimination, and photosynthesis-related traits and the co-localization and clustering of physiological traits with yield was observed in a number of regions, similar to the results observed in this study.

As is the case in many QTL and MQTL studies, regions containing major genes were shown to have a strong influence on trait measurement and subsequently QTL identification.

This result is in strong agreement with Zhang et al. (2010) who showed QTL for plant height to be strongly associated with yield and yield components across 60 studies. In the current study, major genes such as *Ppd-B1*, *Ppd-D1*, *Rht-B1* and *Rht-D1* were all found to lie within a QTL cluster and a MQTL region. However, some additional major genes including the *B1* awn locus on chromosome 5AL and the vernalization genes on the group 5 chromosomes appear to not have been detected as MQTL, despite previous studies showing their association with yield and adaptation. It is possible that the occurrence of segregation for these genes was low in the populations evaluated in the 30 studies or that their effect on yield and other traits is lower than previously thought.

MQTL34 on chromosome 4B was located on the homologous genomic region of rice (chromosome 1) previously identified by Swamy et al. (2011) as a major MQTL associated to drought resistance. *Rht-B1*, one of the two major genes in wheat regulating plant height is also known to be located in this region, but it is unclear whether a homolog might be present within this region in rice. In the current study, this region was also associated with physiological and agronomic trails such as chlorophyll, days to maturity, flag leaf length, Fo, Fv, Fv/Fo, kernel weight, osmotic potential, root length single kernel weight, spike harvest index, and water soluble carbohydrates. These traits had been also used in previous MQTL studies (Li et al. 2012; Zhang et al. 2010). The presence of a large QTL cluster at MQTL5 on chromosome 1BS might prove beneficial for breeders to focus on improvement of traits such as kernel number per spike, yield, drought susceptibility index osmotic potential, and coleoptile length (Peleg et al. 2008; Zheng et al. 2010; Quarrie et al. 1994).

On chromosome 2D, where major genes such as *Ppd- D1* controlling photoperiod (Beales et al. 2007) and *Rht-8* controlling height and gibberellin pathways (Sip et al. 2009) have been

reported, four meta-QTL; MQTL19, MQTL20, MQTL21, and MQTL22, related to both heat and drought stress were identified. Traits such as kernel number, thousand kernel weight, time to maximum fluorescence, green area, stem reserve mobilization, root shoot ratio, days to maturity, flag leaf weight, leaf carbon isotope discrimination, stem water soluble carbohydrates during flowering, yield, and height clustered in these MQTL regions. Stem development inhibition as a result of gibberellin gene action on plant physiology results in more available photoassimilates for grain filling, yield production and quality (Flintham et al. 1997). Moreover, low carbon isotope discrimination has been associated with an increase in transpiration efficiency contributing to the photosynthetic process (associated with green area) increasing yield production (Rebetzke et al. 2006).

Seventy four lines from the Gulf Atlantic Wheat Nursery were evaluated with twenty one SSR markers linked to important MTQL regions identified in this study. These MQTL were located on chromosomes 2B, 2D, 3B, 4A, 4D, 5A, 6B, 7A. A total of 346 alleles were detected for the 21 markers, suggesting there is high level of allelic diversity present within this soft-red winter wheat germplasm, according to the genetic diversity parameters established by Zhang et al. (2002) in order to determine genetic relationships between wheat lines. In general, a high average PIC value (0.83) for the 21 markers and a high correlation coefficient between gene diversity and number of alleles with r = 0.7892 was observed. SSR markers *gwm257*, *gwm156*, *gwm537* and *gwm388* associated with the three MTQL regions identified in this study were in agreement with previous studies identifying QTL related to thousand kernel weight, protein content, carbon isotope discrimination, height, and yield production (Groos et al. 2003; Rebetzke et al. 2008; Mathews et al. 2008). However, further work is needed to determine if the allele sizes of these markers correspond to those present within the GAWN lines and could potentially

be used for marker assisted selection. Markers *gwm484* and *gwm156* on chromosomes 2D and 5A had the highest allelic diversity. It is possible this could be due to the fact that important major genes such as *Ppd-1* on chromosome 2D (Beales et al.2007) and the vernalization gene on chromosome 5A (Sutka et al. 1999) are present in these regions and allelic diversity is high for these genes. The number of QTL and traits presented in this region was also high, with 15 individual QTL and 14 traits identified in the region linked to the marker *gwm48* on 2D and 15 QTL and 15 traits linked to the marker *gwm156.* On the other hand, marker *gwm388* had the lowest PIC value of the markers evaluated (0.62) and was only associated with five QTL. Overall, the high level of genetic variation from the twenty one SSR markers selected from the clustering regions identified in this study is promising for the future development of markers for marker assisted breeding for drought and heat tolerance.

Using traits present within MQTL regions, it was possible to develop Stress Trait Expression Pathways (STEPs) that can be used to dissect the genetic and physiological response of wheat to stress. STEPs are developed by defining the genetic relationship between physiological and agronomic traits from the conceptual model developed by Reynolds et al. (2007). An example is presented in Figure 9. In this example, coleoptile vigor results in increased ground cover and a lower level of soil moisture evaporation. This results in improved transpiration efficiency and photosynthesis, more carbohydrate for grain-filling and ultimately higher yield. Since the modification of one trait present in each MTQL can subsequently enhance or maximize the function of other closely related traits also present in the MQTL, the agronomic quality of the crop can be improved (Reynolds et al, 2000). Trait evaluation and STEP development in each MQTL can be used for further wheat genetic improvement and for making informed decision about marker assisted breeding within these regions.

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Table 1. Description of studies used to develop a QTL database for heat and drought tolerance

Study No.	Target stress	Population	Pop Size	No. of markers	No. QTL	Reference
1	Drought and heat	Tamgurt/Cham1	110 RILs	468	206	Diab et al. (2008)
2	Drought	Oste-Gata/Massara	151 RILs	30	20	Golabadi et al. (2011)
3	Drought	Dharwar Dry/ Sitta	140 RILs	25	1	Kirigwi et al. (2007)
4	Adaptive trait	Jennah Khetifa/ Cham1	110 RILs	301	3	Kubo et al. (2007)
5	Drought and heat	Trident /Molineux	182 DHs	260	2	Kuchel et al. (2007a, 2007b)
6	Drought	Opata 85/ W7984	114 RILs	>1000	33	Landjeva et al. (2008)
7	Drought	Huapei3/ Yumai57	168 DHs	324	30	Liang et al. (2010)
8	Drought	Kofa/Svevo	249 RILs	232	42	Maccaferri et al. (2008)
9	Heat	Halberd/ Cutter	64 RILS	170	27	Mason et al (2010)
10	Heat	Halberd/ Karl92	121 RILs	189	14	Mason et al. (2011)
11	Drought	SeriM82/ Babax	194 RILs	587	16	Mathews et al (2008)
12	Drought and heat	SeriM82/ Babax	194 RILs	587	104	McIntyre et al. (2010)
13	Heat	Kauz/ MTRWA116	144 RILs	81	3	Mohammadi et al (2008)
14	Drought	Langdon/G18-16	152 RILs	690	110	Peleg et al (2009)
15	Drought and heat	SeriM82/Babax	167 RILs	401	104	Pinto et al (2010)
16	Drought	Chinese Spring/SQ1	96 DHs	567	17	Quarrie et al (2005)
17	Adaptive trait	Various	Various	Various	21	Rebetzke et al (2008)
18	Adaptive trait	Various	Various	Various	34	Rebetzke et al. (2007)
19	Drought	W7984/ Opata85	114 RILs	2150	3	Salem et al. (2007)
20	Drought	Spark/Rialto	144 RDH	NR	5	Foulkes et al (2007)
21	Drought	Beaver/ Soissons	34 DHs	241	7	Verma et al (2004)
22	Heat	Ventnor/ Karl92	101 RILs	450	16	Vijayalakshmi et al (2010)
23	Drought	Beaver/ Soissons	46 DHs	126	8	Weightman et al (2008)
24	Drought	Hanxuan10/Lumai 14	150 DHs	395	5	Wu et al (2010)
25	Heat	Ventnor/ Karl92	166 F2	59	2	Yang et al. (2002)
26	Drought	Hanxuan10/Lumai 14	150 DHs	395	110	Yang et al (2007a)
27	Drought	Hanxuan10/Lumai 14	150 DHs	395	11	Yang et al (2007b)
28	Adaptive trait	Wangshuibai/ Wheaton	139 RILs	>248	6	Yu et al (2010)
29	Adaptive trait	W7984/ Opata85	114 RILs	2150	16	Zhang et al. (2010)
30	Drought and heat	Arche/Recital	222 DHs	182	131	Zheng et al. 2010

Table 2. Pedigree diversity lines study.

ID	PEDIGREE
NC09-22206	NC00-16203 // P26R24 / NC96-13965
NC09-21256	NC00-16203 // P26R24 / NC96-13965
NC09-20765	NC00-16203 // P26R24 / NC96-13965
NC09-20768	NC00-16203 // P26R24 / NC96-13965
NC09-19966	NC00-16203 // P26R24 / NC96-13965
NC09-21230	NC00-16203 // P26R24 / NC96-13965
NC09-19946	NC00-16203 // P26R24 / NC96-13965
NC09-21251	NC00-16203 // P26R24 / NC96-13965
NC09-22402	NC99-18235 / NC00-16203 // Dominion
NC09-21953	NC99-18235 / NC00-16203 // Dominion
NC08-140 (Bdv2)	P26R61 / TC14Spear 2289B // NC00-16203
NC09-20932	Trego / NC99BGTAG11 // NC98-13296W
USG 3555*	VA94-52-60/Pioneer Brand ‘2643’ //‘USG 3209’. USG 3555
FL04363E-P19	FL03155 F1(VA01W-112/FL9547)/AGS 2060
LA03045E-4	LA95361CA18-1/LA95176D56-2
LA03091E-63	LA97113UC-124-3(Ceruga15//LA85411-D4,FR81-19/SALUDA)/PIO26R61
LA03200E-2	NC98-24710/PIO26R61
LA04013D-111	LA95135/B990399
LA04041D-85	AGS2060/GA951079A25
LA05038D-105	SS8641/P26R61
LA05038D-51	SS8641/P26R61
LA05130D-P5	LA98149BUB-3-4-B(FL931339AS/P26R61)/SS8641
LA05132F-P09	LA98149BUB-3-4-B(FL931339AS/P26R61)/AGS2060
LA06007E-P04	APCK B02-8443/LA95135
LA06052E-P07	SS8641/LA95135
AGS2060*	FL-302/FR-81-19(GA-85430-D-17-2-P-1)//CK-9663[2965]
SS8641*	GA 881130 / 2* GA 881582'// GA 881130 'KSH8998 / FR 81-10 //
GA041052-11E51	931233-28-2-2 / USG3592
GA041293-11E54	P26R61 / 96229-7-4-4 // 96229-3A41
GA04244-11E1	96229-3A39 / AGS 2000
GA04417-11E21	961565-2E46 / AGS2485 // 96229-3A41
GA041323-11E63	95652-2E56 / 961591-3E42
GA04151-11E26	98302-17-1-4 / SC996284
GA04434-11E44	961565-2E46 / AGS2485 // 96229-3A41
GA041293-11LE37	P26R61 / 2*SS 8641
GA04444-11LE25	96229-3A41 / 951395-3E27
SCLA1110R1	P26R61/NC98-24710

Table 2. Pedigree diversity lines study (Cont.)

ID	PEDEGREE
GA04510-11LE24	961591-3E42 / 96229-3A41
GA041296-11LE39	McCormick / 961591-17-1-5 // 951395-3A31
GA04500-11LE11	97531-2-11 / 011636-G1-G5-G2 (A2000*3 / 93322)
AR00179-4-1	IL94-6727 x Roane
AR00343-5-1	AR97052 x Roane
AR00380-3-3	AR97054 x 97201
AR01039-4-1	AR800-1-3-1/AR839-28-1-2
AR01040-4-1	AR800-1-3-1/AR910-12-1
AR01044-1-1	AR800-1-3-1/AR92145E8-7-7-1-0
AR01058-1	AR839-27-1-3/ Roane
AR01156-2-1	UGA 901146E15/AR839-27-1-3
AR01168-3-1	VA 98W-593/AR839-28-1-2
AR01177-2-1	AR92145E8-7-7-1-0/AR9035-4-2
AR01205-1-1	PI155271/ARLA85411
AR01209-2-1	AGS2000/PI531193 (JGI)
VA07W-415	VA98W-895 / GA881130LE5 // VA98W-627RS
VA09W-46	GF921221E16 / McCormick"S" // VA99W-200
VA09W-52	GF921221E16 / McCormick"S" // VA99W-200
VA09W-110	USG 3592 (GA931241E16) / VA01W-303
VA09W-112	USG 3592 (GA931241E16) / VA01W-303
VA09W-69	SS 520(VA96W-158) / VA99W-188 // Tribute
VA09W-114	USG 3592 (GA931241E16) / VA01W-303
VA10W-123	Pioneer 25R47 / GF951079-2E31
VA10W-125	Pioneer 25R47 / Jamestown
VA10W-140	VA01W-210 / SS 520 (VA96W-158) // Tribute
VA08W-613	Freedom / Neuse"S" // VA98W-688
VA10W-663	P97397B1-4-5 / McCormick // Coker 9511
SCLA1030J1	LA94162D157-1(FR93.13/Morey SIB)/AGS2000
SCLA1067A1	KS94U275/AGS2000
SCLA1084A1	NC98-24710(HFL)/AGS2000
SCLA1084B1	NC98-24710(HFL)/AGS2000
SCLA1084C1	NC98-24710(HFL)/AGS2000
SCLA1084K1	NC98-24710(HFL)/AGS2000
SCLA1102D1	P26R61/LA841
SCLA1102G1	P26R61/LA841
SCLA1102G3	P26R61/LA841
SCLA1102H1	P26R61/LA841
SCLA1110P1	P26R61/NC98-24710

Table 3. Selected MTQL, chromosome, and linked SSR used for allelic diversity analysis

MTQL identified	Chromosome	Linked SSR
MQTL2	1A	wmc312
MQTL6	1B	wmc766
MQTL4	1B	wmc406
MQTL5	1B	gwm498/gwm18
MQTL13	2A	gwm356
MQTL15	2B	gwm257
MQTL17	2B	gwm388
MQTL19	2D	gwm102
MQTL18	2D	gwm484
MQTL26	3B	gwm108
MQTL25	3B	barc164
MQTL32	4A	barc78
MQTL29	4A	wmc680
MQTL33	4B	wmc238
MQTL34	4D	barc91
MQTL36	5A	barc117
MQTL37	5A	gwm156
MQTL50	6B	gwm70
MQTL57	7A	wmc607
MQTL59	7B	gwm537

Table 4. Physiological and agronomic traits associated with drought and heat tolerance

Trait association	Abbreviation
% Screening	%scr
a:b peak height ratio	PHR
Accumulation efficiency of Stem water soluble carbohydrates	ASSC
Biomass	BIO
Biomass per spike	BSPK
Biomass production rate	RBIO
Canopy temperature during grain fill	CTgf
Canopy temperature during vegetative stage	CTv
Carbon isotope discrimination	GCID
Chlorophyll	CHL
Coleoptile cross-sectional area (mm)	COLA
Coleoptile length (mm)	COLL
Coleoptile spiraling (mm)	COLS
Culm length	SPKL
Days to heading	DTH
Days to maturity	DTM
Drought susceptibility index Carbon isotope discrimination	dCID
Drought susceptibility index Chlorophyll	dCHL
Drought susceptibility index culm length	dSPKL
Drought susceptibility index Days to heading	dDTH
Drought susceptibility index Days to maturity	dDTM
Drought susceptibility index Leaf rolling	dROLL
Drought susceptibility index osmotic potential	dOP
Drought susceptibility index Total dry matter	dBIO
Drought susceptibility index yield	dYLD
Dry mass accumulation culm jointing	SPKB
Dry mass accumulation leaves at anthesis	LFB
Dry mass accumulation plants jointing	BIO
Flag leaf length	FLL
Flag leaf width	FLW
Fm	Fm
Fo	Fo
Fv	Fv
Fv/Fm at anthesis	Fvm
Fv/Fo	Fvo
Grain fill rate	GFR
Grain filling efficiency early	GFE
Grain Hardness	KHA
Grain-filling duration	GFD
Green leaf area	SG
Harvest index	HI
Height	HGT
HSI Grain-filling duration	hGFD
HSI single kernel weight	hSKW
Kernel number	KN

Table 4. Physiological and agronomic traits associated with drought and heat tolerance(Cont.)

Trait association	Abbreviation
Kernel number per spike	KNS
Kernels per m2	Km2
Leaf Carbon isotope discrimination	LCID
Leaf rolling	ROLL
Leaf temperature depression	LTD
Maximum rate of senescence	MRS
Normalized difference vegetative index during grain fill	NDVIg
Normalized difference vegetative index during vegetative	NDVIv
Normalized difference vegetative index vegetative stage	NDVIv
Osmotic potential	OP
Protein content	PRO
PVRN number of roots penetrating through the PV disc by each plant	RTV
Remobilization efficiency of Stem water soluble carbohydrates	ESSC
Root dry weight	RBIO
Root length	RTL
Root length tolerance index	sRTL
Root penetration index	RTV
Root to shoot ratio	R:S
Shoot length	HGT
Shoot length tolerance index	sHGT
Single kernel weight	SKW
Spike dry matter	BSPK
Spike harvest index	HIs
Spike per m2	SM2
Spike temperature depression	LTD
Stem reserve mobilization	MWSC
Stem water soluble carbohydrates	SSC
Stem water soluble carbohydrates at maturity	SSCm
Stem water soluble carbohydrates during flowering	SSCa
Stem water soluble carbohydrates during grain-fill	SSCg
Stress susceptibility index of kernel weight	hKW
Test weight	TW
Thousand kernel weight / Thousand kernel weight at maturity	TKW
Thousand kernel weight at grain fill	TKWg
Time to maximum rate of senescence	TMRS
Tm (Time to max fluorescence)	Tm
Total dry matter	BIO
Visual leaf wax	WAX
Vitreosity	VIT
Water soluble carbohydrate area basis	WSC
Yield	YLD

Table 5. Description of MQTL detected for heat and drought stress

MQTL No.	Chr.	No. QTL	No. of traits	No. of studies	Position (cM)	CI (cM)	Flanking markers	Stress
MQTL1	1A	2	2	2	35.32	15.94	gwm33-barc83	Drought
MQTL2	1A	13	11	6	60.11	4.05	wmc744-wmc826	Drought
MQTL3	1A	4	4	3	89.43	11.55	wmc9-wmc59	Drought
MQTL4	1B	13	12	5	23.56	2.80	wmc619-barc8	Mostly drought
MQTL5	1B	23	17	13	34.22	2.12	cfd2-wmc213	Drought and heat
MQTL6	1B	10	6	4	63.00	5.46	wmc206-cfa2147.1	Drought and heat
MQTL7	1B	4	3	4	110.46	6.78	gwm259-gwm140	Adaptive
MQTL8	1D	5	5	4	29.63	13.59	wmc432-gwm106	Mostly drought
MQTL9	1D	5	6	4	57.07	4.56	barc148 -cfd19	Mostly drought
MQTL10	2A	4	4	2	9.13	5.69	gwm512 - gwm614	Mostly heat
MQTL11	2A	8	6	4	55.52	7.46	wmc474 - gwm372	Drought
MQTL12	2A	4	4	2	69.98	7.16	gwm47-gwm312	Drought and heat
MQTL13	2A	11	7	4	126.00	2.84	gwm356-barc76	Mostly heat
MQTL14	2B	7	4	2	10.27	4.03	wmc382-barc124	Drought
MQTL15	2B	11	6	4	37.70	4.29	wmc213-wmc770	Mostly heat
MQTL16	2B	6	4	2	48.95	3.85	gwm148-barc7	Drought
MQTL17	2B	15	14	4	68.00	2.06	gwm55-gwm388	Mostly drought
MQTL18	2B	5	4	3	101.17	3.74	wmc149-wmc361	Drought
MQTL19	2D	15	8	5	41.00	2.75	wmc470-wmc453	Drought/heat
MQTL20	2D	13	4	3	47.95	2.99	barc168-gwm102	Drought and heat
MQTL21	2D	3	3	2	64.00	3.32	cfd2-barc145	Drought
MQTL22	2D	6	4	3	90.93	15.98	barc228-gwm383	Drought
MQTL23	3A	2	2	2	0.80	7.64	wmc11-wmc532	Drought
MQTL24	3A	2	2	2	45.39	19.63	gwm2-wmc428	Drought/heat
MQTL25	3B	9	7	6	5.83	2.53	wmc674-barc147	Drought/heat
MQTL26	3B	11	9	6	69.30	4.86	wmc307-wmc148	Mostly drought
MQTL27	3B	10	10	6	93.98	4.30	wmc291-barc206	Drought/heat
MQTL28	3B	6	6	3	138.83	4.25	gwm547-wmc274	Drought/heat
MQTL29	3D	7	7	4	25.64	1.85	gdm136-gwm52	Drought
MQTL30	4A	24	19	9	7.89	1.25	wmc420-wmc173	Drought/heat
MQTL31	4A	7	5	6	35.94	6.97	barc170-wmc707	Drought/heat
MQTL32	4A	8	6	3	60.99	3.37	wmc283-wmc500	Mostly drought
MQTL33	4A	11	8	5	74.71	5.06	barc70-gwm160	Drought/heat
MQTL34	4B	32	17	11	24.71	2.01	wmc254-wmc546	Drought/heat
MQTL35	4D	18	15	6	32.50	2.90	cfd106-wmc182	Drought/heat
MQTL36	4D	3	3	2	82.00	2.64	gwm194-wmc74	Heat
MQTL37	5A	4	4	2	32.04	10.05	wmc713-wmc654	Drought/heat
MQTL38	5A	14	14	7	57.44	2.63	gwm415-wmc805	Drought/heat
MQTL39	5A	16	11	11	72.54	2.68	barc40-gwm639	Drought/heat
MQTL40	5A	7	6	3	140.16	4.27	gwm126-wmc577	Drought/heat

Table 5. Description of MQTL detected for heat and drought stress (Cont.)

MQTL No.	Chr.	No. QTL	No. of traits	No. of studies	Position (cM)	CI (cM)	Flanking markers	Stress
MQTL41	5A	5	3	4	165.70	5.01	gwm291-B1	Mostly heat
MQTL42	5B	3	2	2	57.00	5.16	gdm146-wmc682	Drought
MQTL43	5B	7	6	7	67.08	2.70	gwm67-wmc435	Drought/heat
MQTL44	5B	7	7	3	117.00	3.26	wmc75-wmc810	Mostly drought
MQTL45	5B	3	3	2	135.15	3.71	gdm116-wmc235	Drought/heat
MQTL46	5D	3	3	3	25.97	3.72	barc143-cfd81	Drought
MQTL47	5D	9	9	4	64.30	7.54	wmc289-cfd156	Drought/heat
MQTL48	6A	7	6	3	1.95	3.75	gwm459-gwm334	Mostly drought
MQTL49	6A	5	2	2	83.92	7.97	gwm169-wmc417	Mostly heat
MQTL50	6A	4	3	3	93.05	5.33	wmc580-wmc642	Drought
MQTL51	6B	5	4	3	27.00	5.08	cfd13-gwm508	Drought
MQTL52	6B	14	11	6	42.10	3.69	wmc397-barc198	Drought/heat
MQTL53	6B	3	2	3	59.83	9.30	barc24-wmc417	Drought
MQTL54	6D	3	3	3	22.98	3.56	gdm132-gwm469	Drought/heat
MQTL55	6D	3	3	2	53.26	6.04	cfd19-gwm55	Drought/heat
MQTL56	6D	2	2	2	110.00	16.10	barc96-wmc773	Drought
MQTL57	7A	2	2	2	4.52	10.52	gwm666-gwm350	Heat
MQTL58	7A	5	5	3	30.10	6.39	wmc168-wmc179	Drought mostly
MQTL59	7A	19	15	8	5.04	3.01	wmc65-wmc607	Drought/heat
MQTL60	7A	9	8	5	100.00	5.58	cfd20-gwm63	Drought/heat
MQTL61	7B	12	11	2	35.11	1.55	wmc76-CFA2106	Drought
MQTL62	7B	7	5	4	56.73	9.32	wmc182-barc95	Drought/heat
MQTL63	7B	5	5	4	138.02	6.83	wmc613-wmc166	Mostly drought
MQTL64	7D	3	3	2	51.00	9.00	cfd41-wmc606	Drought
MQTL65	7D	5	5	4	84.77	8.56	cfd21-gwm111	Drought/heat
MQTL66	7D	2	2	2	141.00	9.90	gwm426-wmc166	Drought

Table 6. Genetic diversity analysis of markers linked to important meta QTL

MQTL Identified	Marker	Chr.	Major Allele Frequency	Sample Size	Allele No	Gene Diversity	PIC
MQTL2	wmc312	1A	0.1622	74	22	0.9142	0.9083
MQTL4	wmc406	1B	0.2297	74	14	0.8682	0.8556
MQTL6	wmc766	1B	0.2568	74	23	0.8831	0.8746
MQTL5	gwm498	1B	0.2027	74	18	0.8828	0.8721
MQTL5	gwm18	1B	0.3514	74	12	0.7838	0.7562
MQTL13	gwm356	2A	0.2973	74	8	0.7984	0.7697
MQTL15	gwm257	2B	0.3108	74	10	0.8093	0.7855
MQTL17	gwm388	2B	0.5270	74	7	0.6578	0.6196
MQTL19	gwm102	2D	0.2973	74	10	0.8240	0.8028
MQTL18	gwm484	2D	0.1081	74	24	0.9361	0.9324
MQTL26	gwm108	3B	0.1622	74	20	0.9112	0.9047
MQTL25	barc164	3B	0.2568	74	17	0.8744	0.8638
MQTL29	wmc680	4A	0.2162	74	18	0.8923	0.8837
MQTL32	barc78	4A	0.1757	74	23	0.9153	0.9096
MQTL33	wmc238	4B	0.4189	74	11	0.7239	0.6852
MQTL34	barc91	4D	0.4054	74	20	0.7973	0.7838
MQTL36	barc117	5A	0.2297	74	13	0.8598	0.8451
MQTL37	gwm156	5A	0.1081	74	21	0.9386	0.9352
MQTL50	gwm70	6B	0.2568	74	13	0.8455	0.8284
MQTL57	wmc607	7A	0.2568	74	22	0.8634	0.8508
MQTL59	gwm537	7B	0.1892	74	20	0.9120	0.9059
Mean			0.2580	74	16.47	0.8520	0.8368

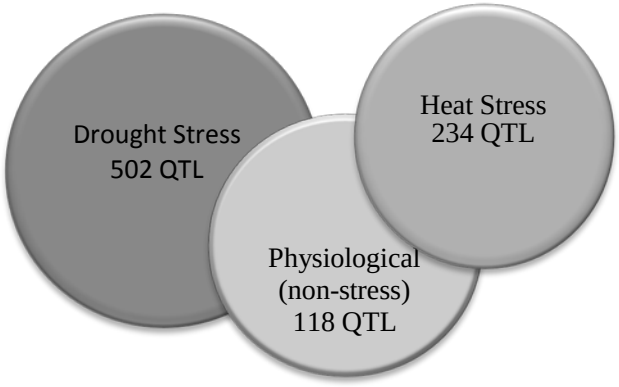


Fig. 1. Number of individual QTL for drought heat and physiological traits

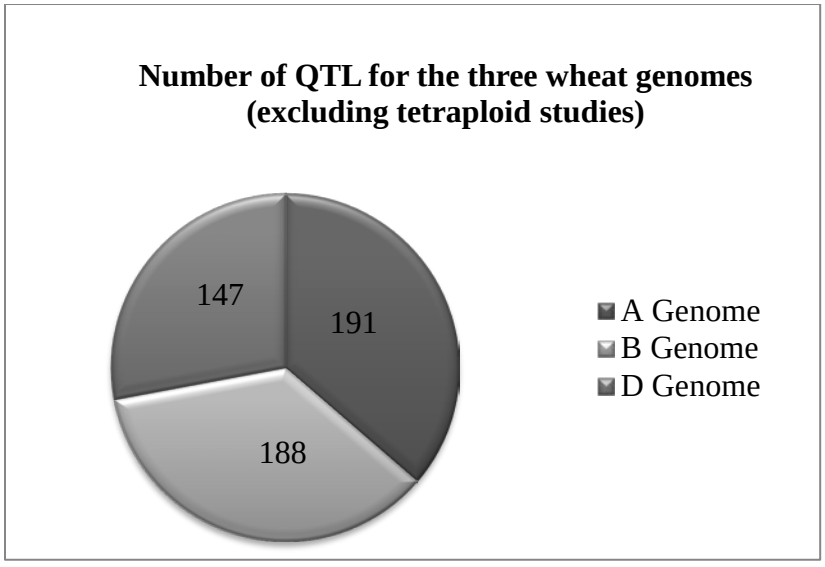


Fig. 2. Number of QTL for the three wheat genomes

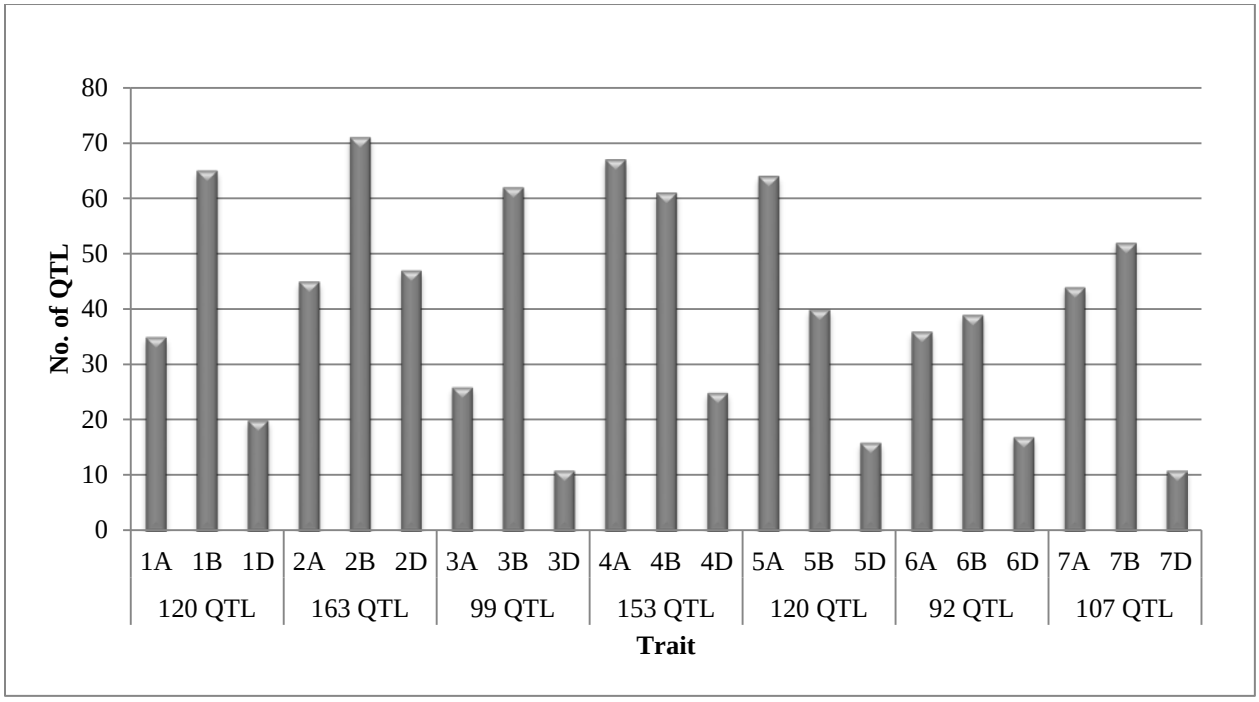


Fig. 3. QTL distribution across 21 wheat chromosomes

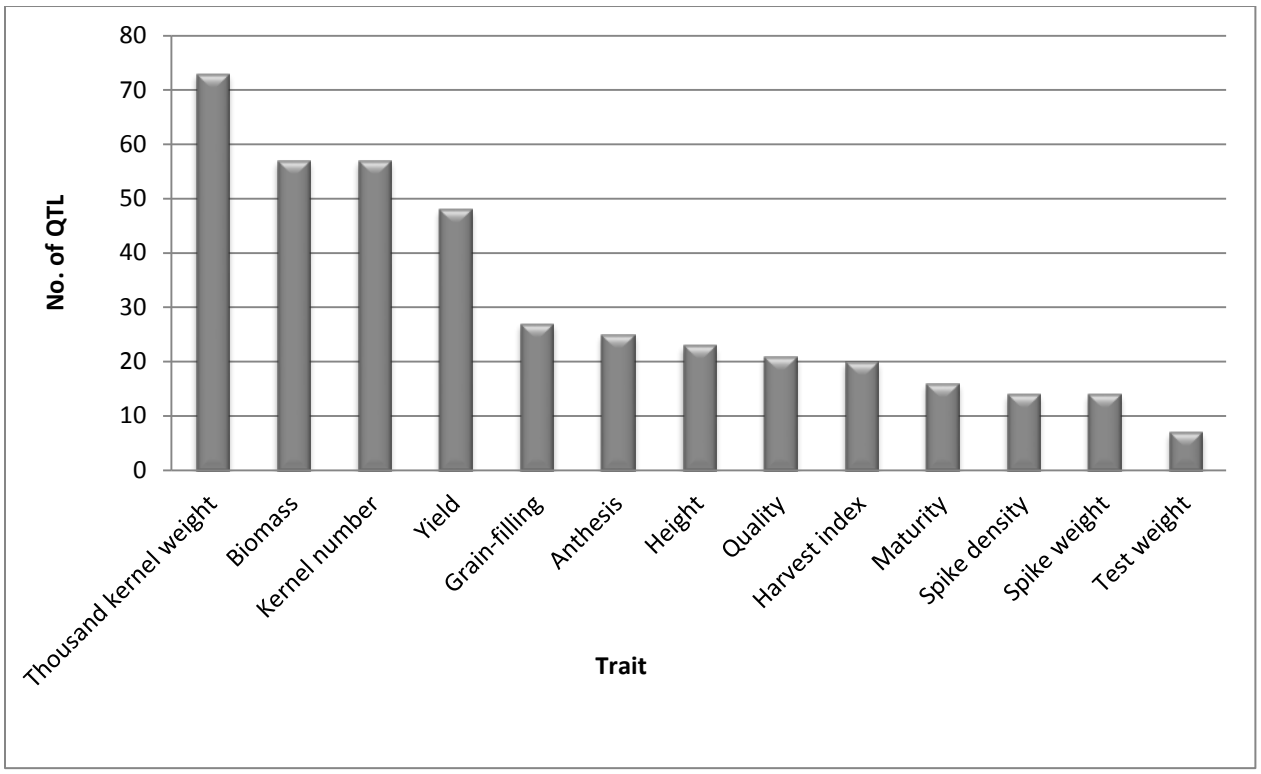


Fig. 4. Summary of agronomic trait QTL.

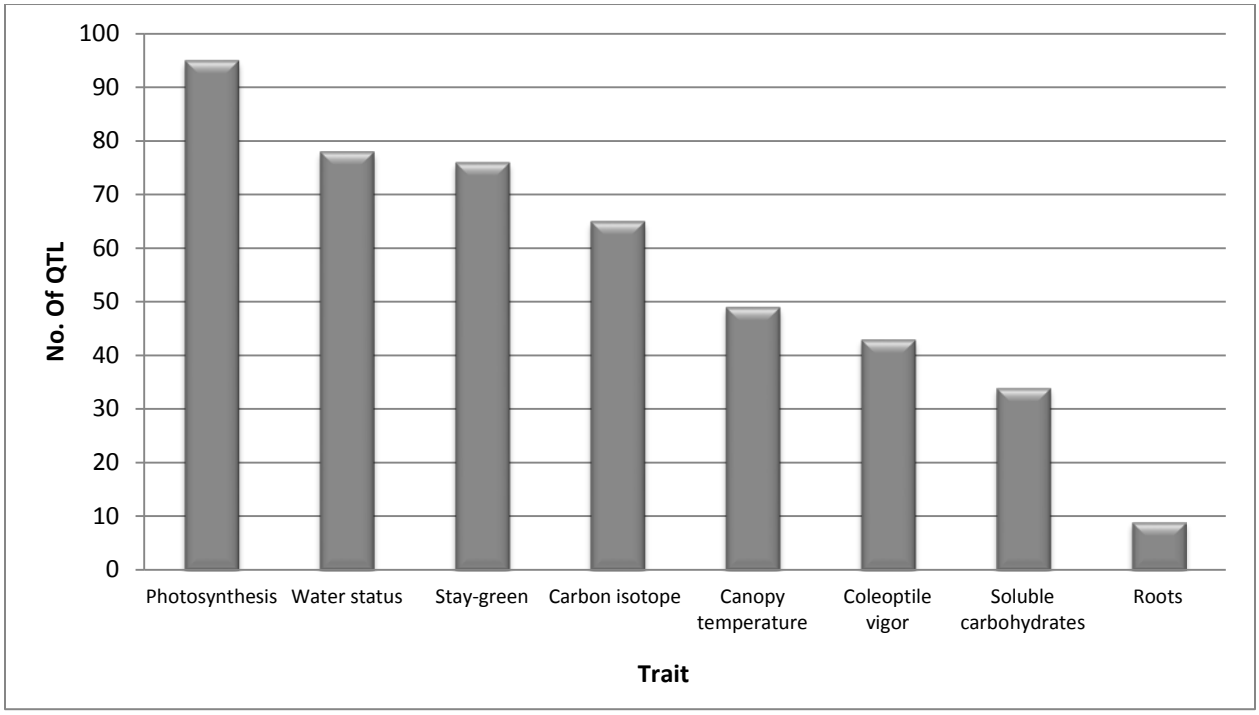


Fig. 5. Summary of physiological trait QTL

Fig. 6. MQTL detected across the 21 wheat chromosomes

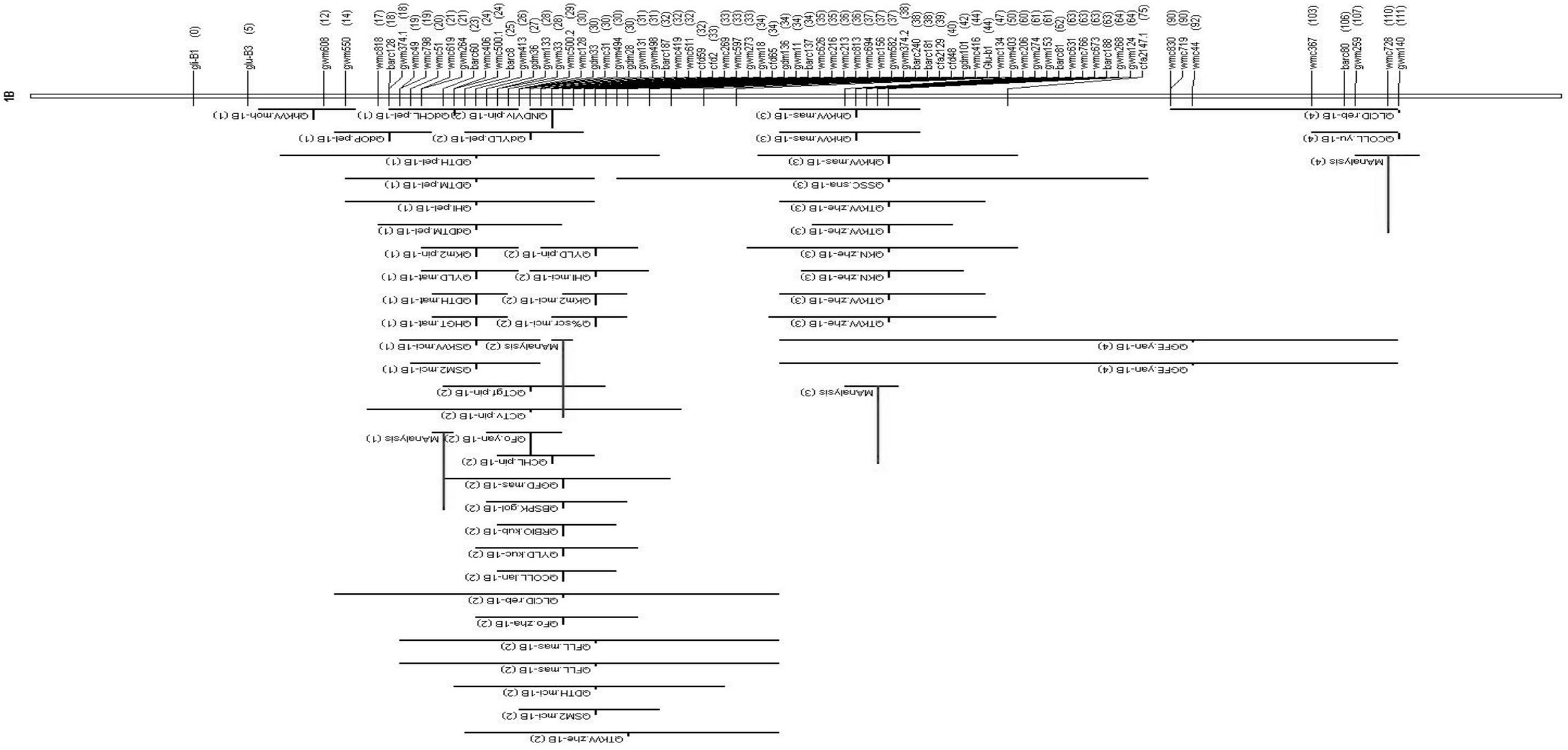


Fig. 6. Continued

Fig. 6. Continued

Fig. 6. Continued

Fig. 6. Continued

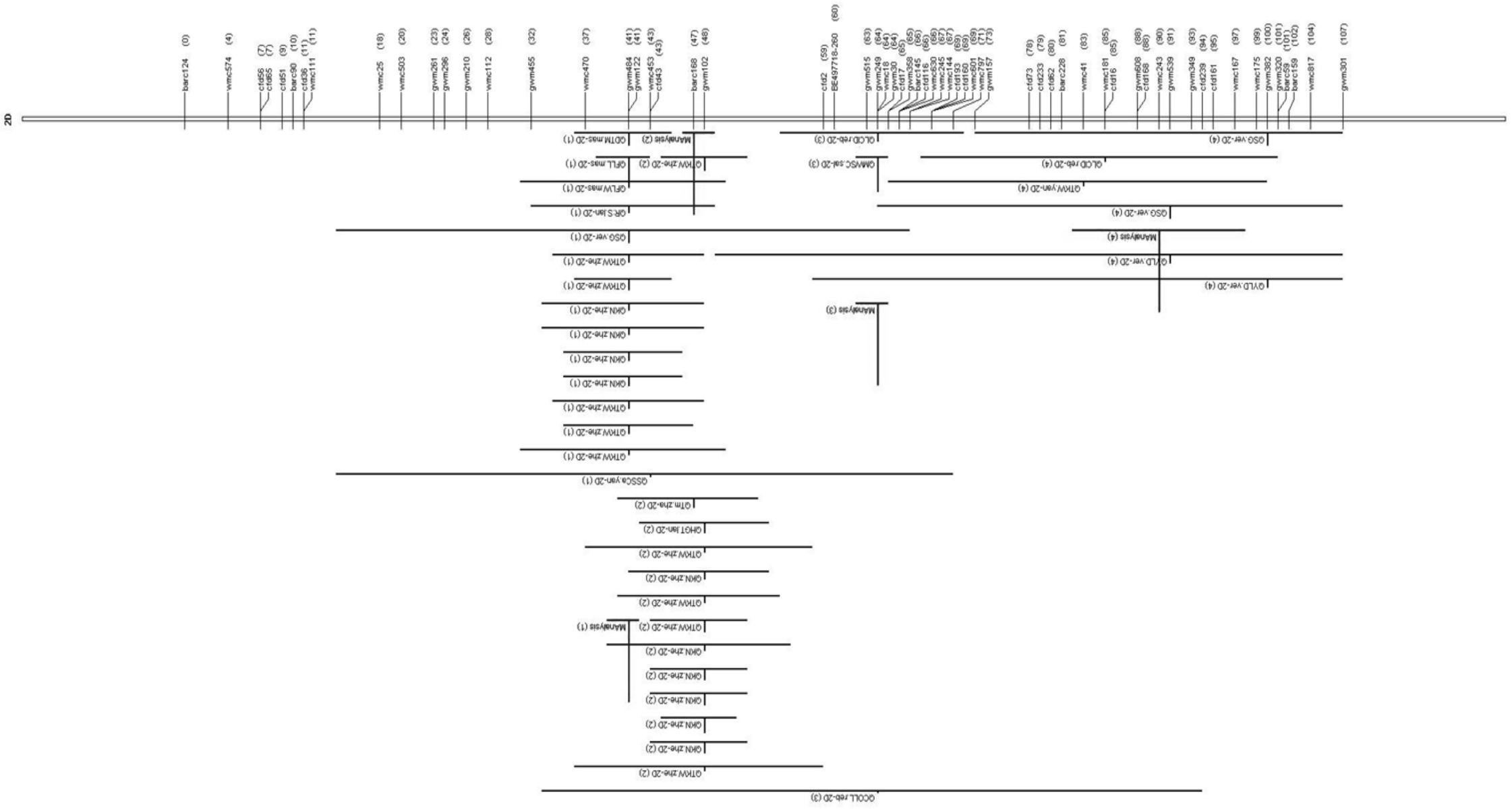


Fig. 6. Continued

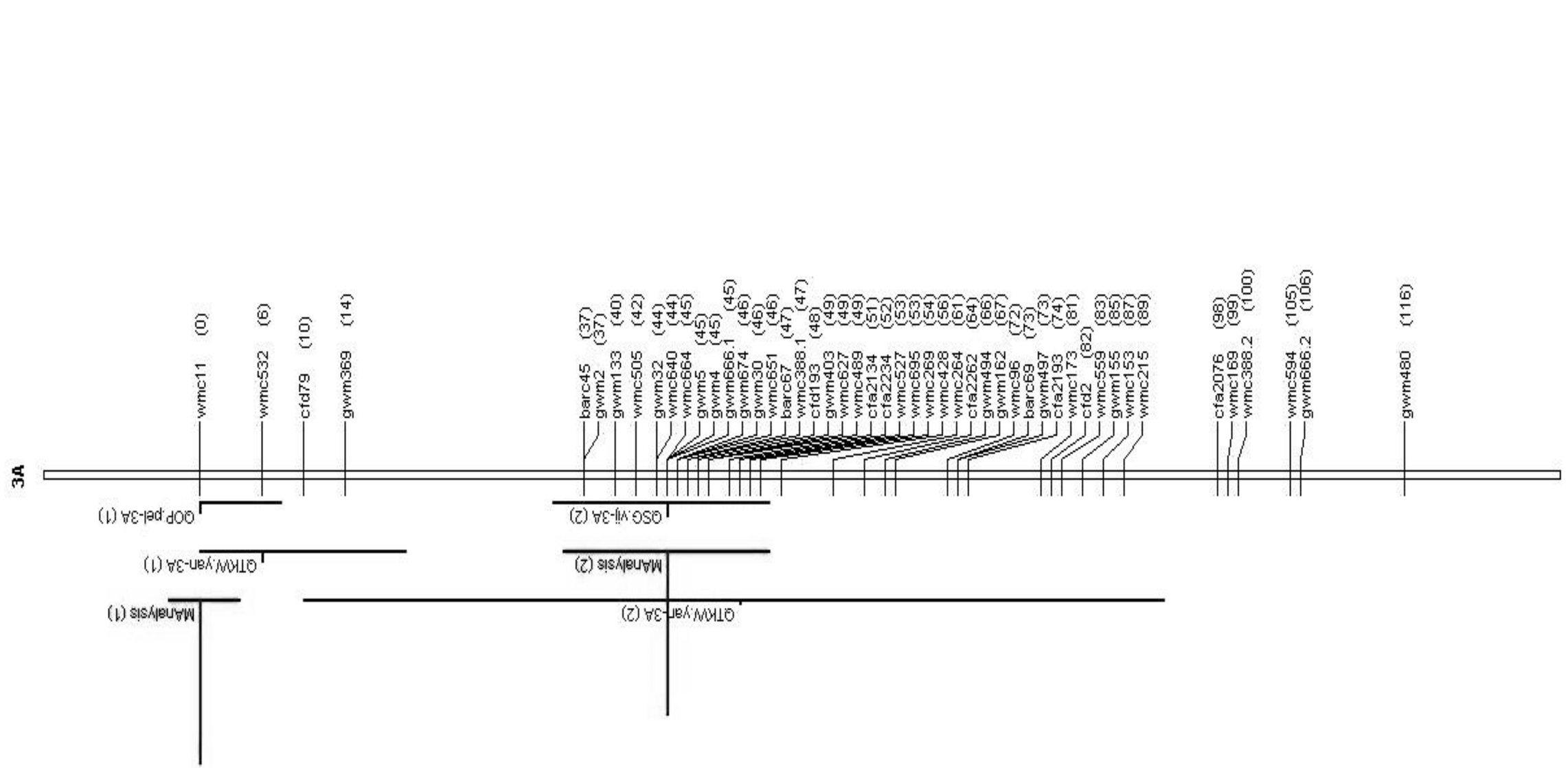


Fig. 6. Continued

Fig. 6. Continued

Fig. 6. Continued

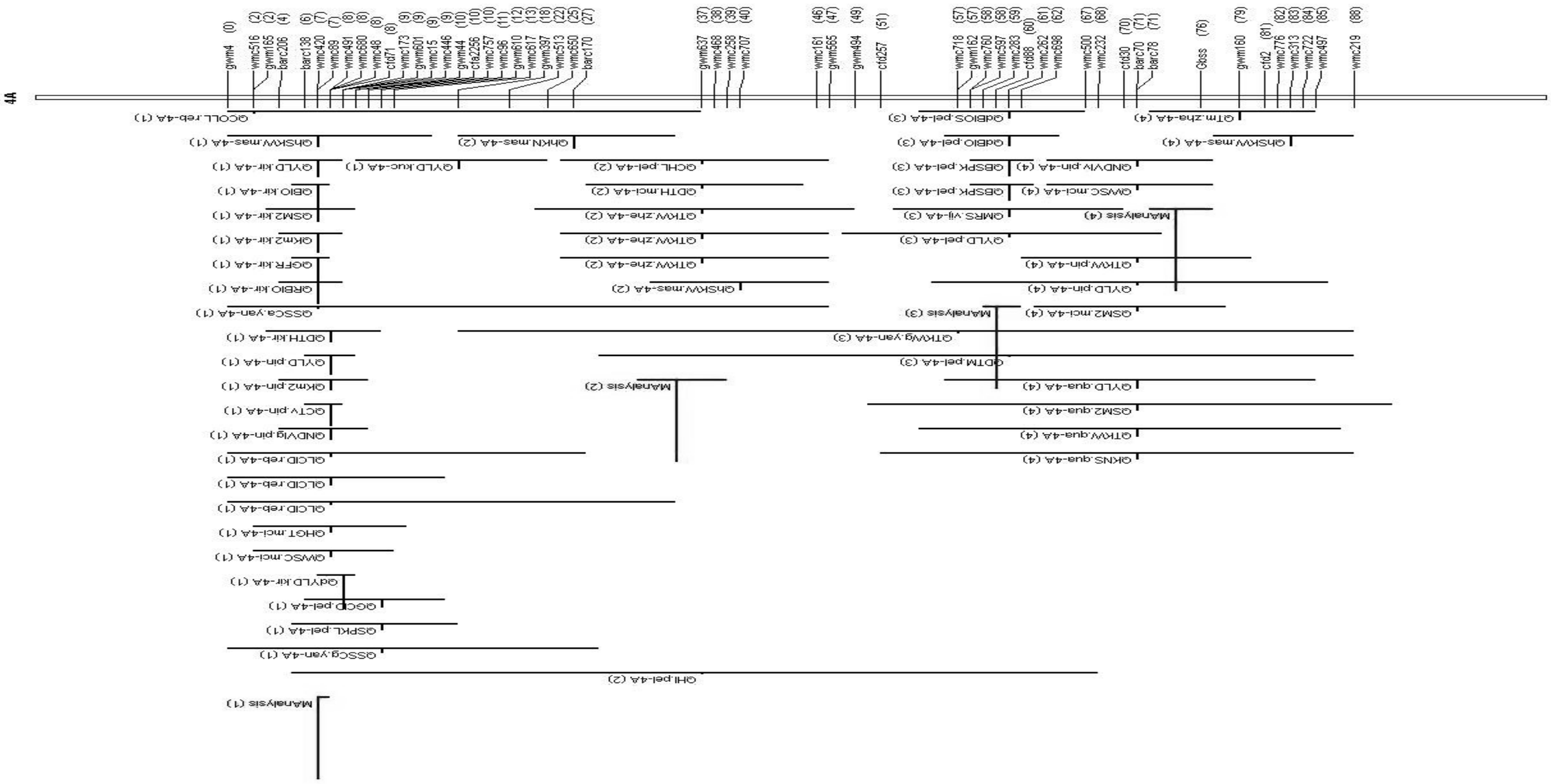


Fig. 6. Continued

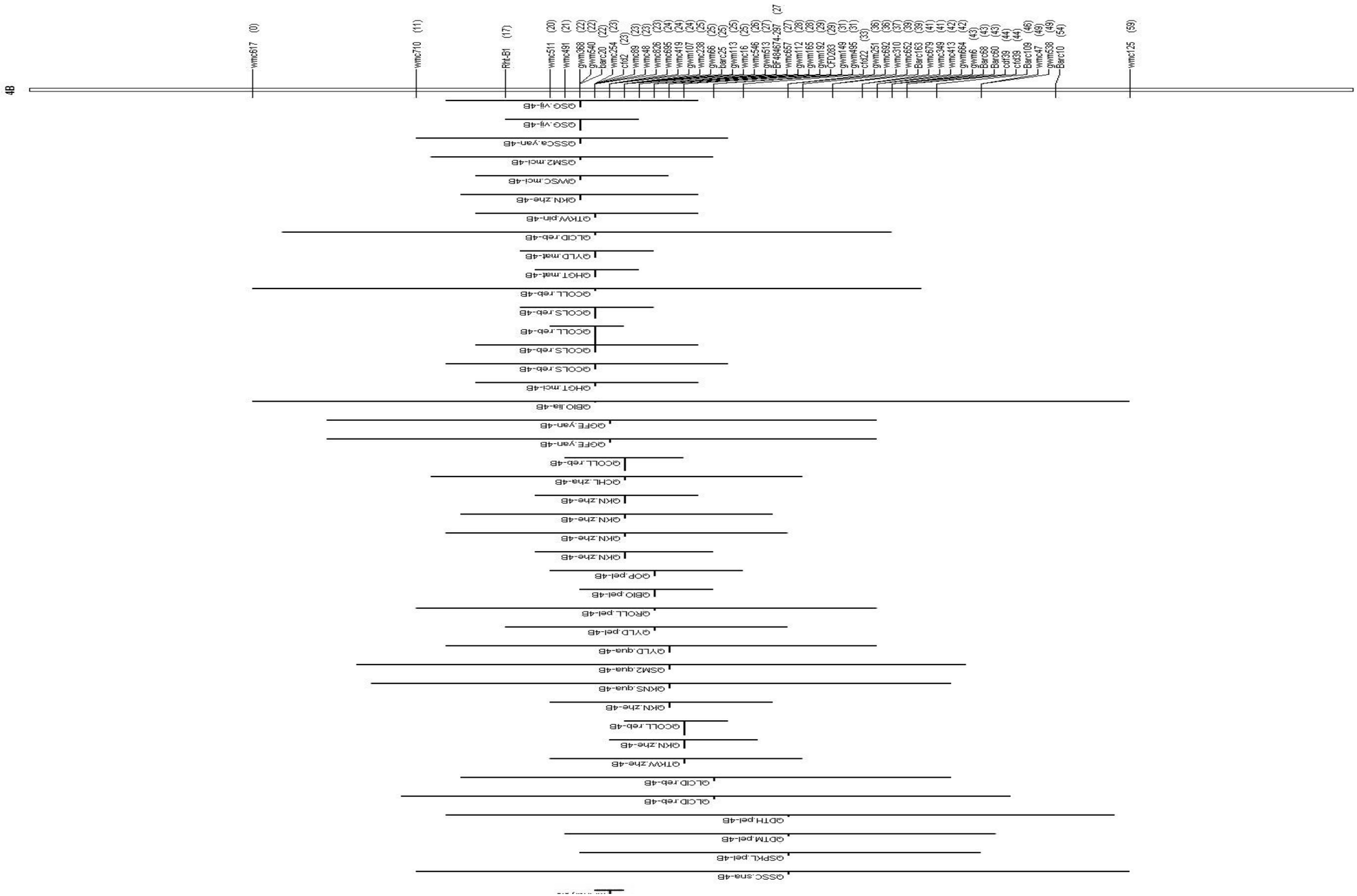


Fig. 6. Continued

Fig. 6. Continued

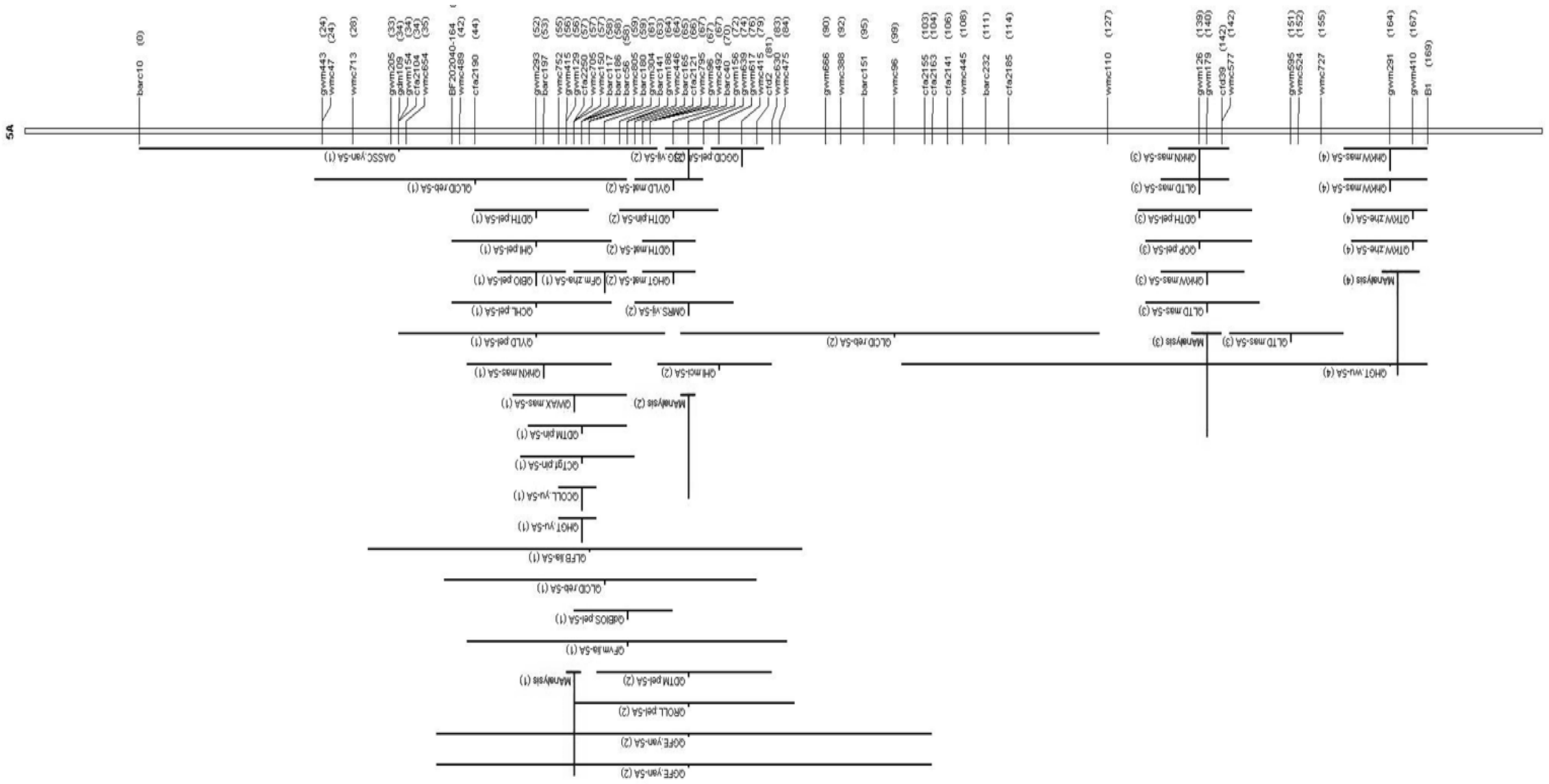


Fig. 6. Continued

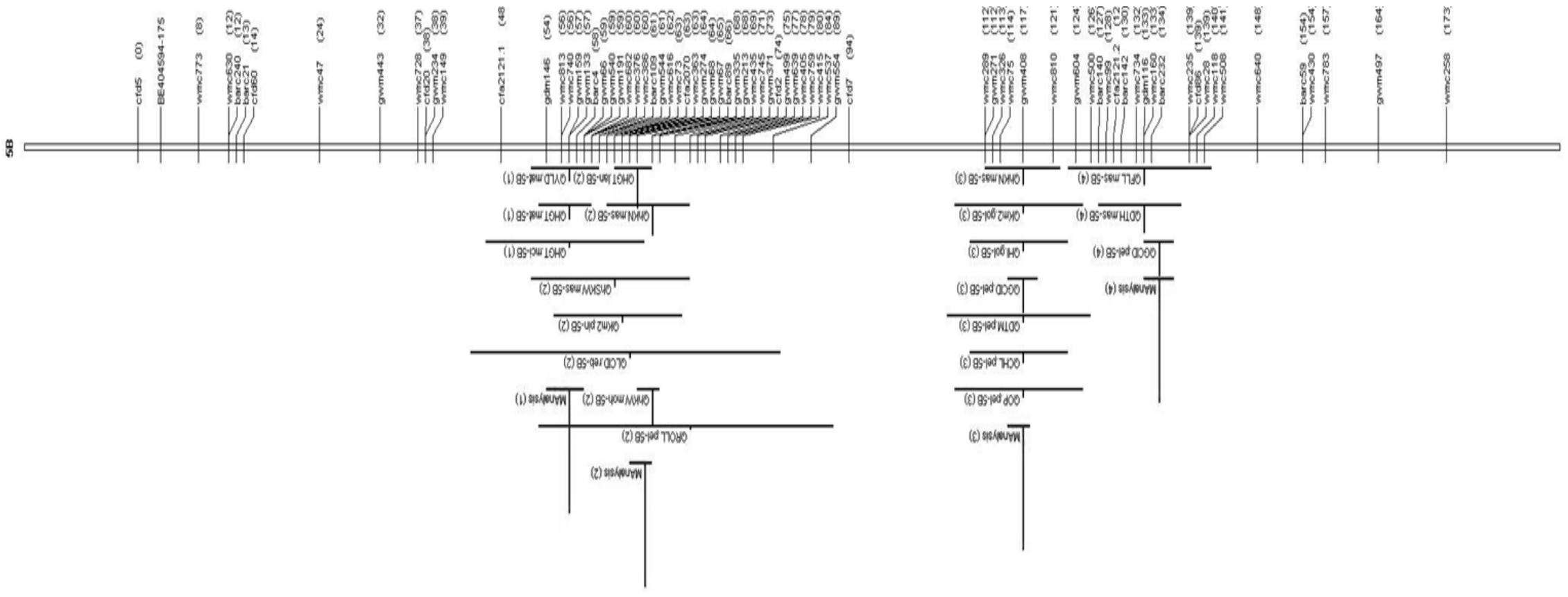
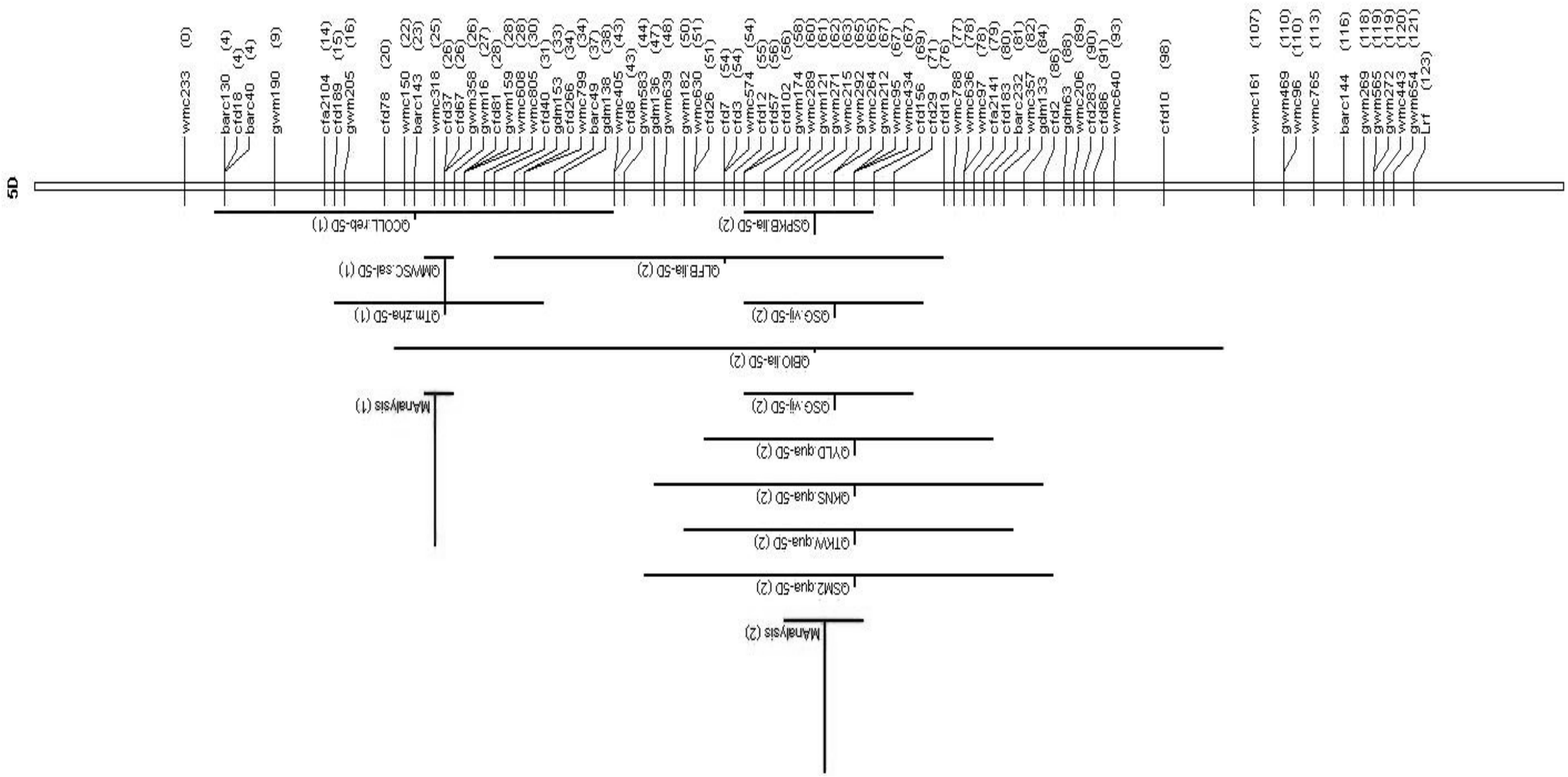


Fig. 6. Continued



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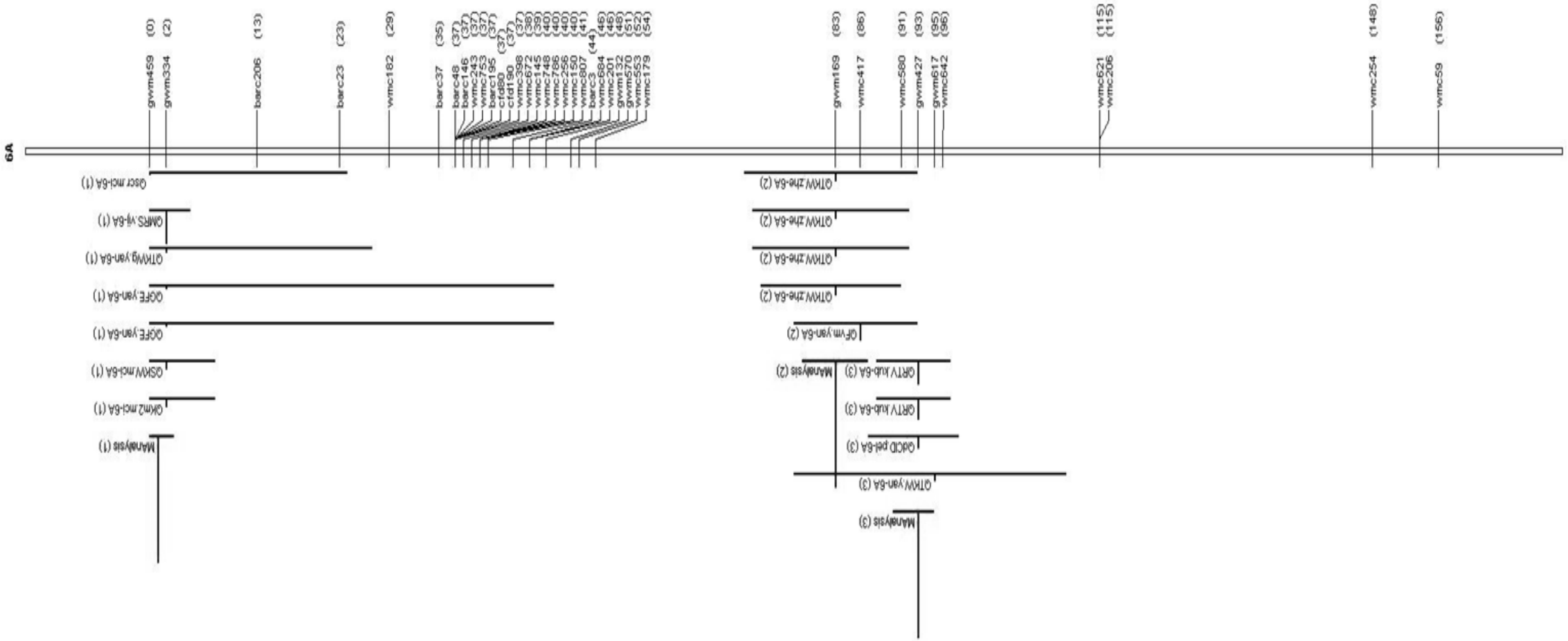
6A
gwm459 (0)
gwm334 (2)
barc206 (13)
barc23 (23)
wmc182 (29)
barc37 (35)
barc48 (37)
barc146 (37)
wmc243 (37)
wmc753 (37)
barc195 (37)
cfd80 (37)
cfd190 (37)
wmc398 (37)
wmc672 (38)
wmc145 (39)
wmc748 (40)
wmc786 (40)
wmc256 (40)
wmc59 (40)
barc807 (41)
barc3 (44)
wmc684 (46)
wmc201 (46)
gwm132 (48)
gwm570 (51)
wmc553 (52)
wmc179 (54)
gwm169 (83)
wmc417 (86)
wmc580 (91)
gwm427 (93)
gwm617 (95)
wmc642 (96)
wmc621 (115)
wmc206 (115)
wmc254 (148)
wmc59 (156)
Qscr.mci-6A (1)
QMRS.vji-6A (1)
QTKW.zhe-6A (2)
QTKW.zhe-6A (2)
QTKW.zhe-6A (2)
QTKW.zhe-6A (2)
QFvm.yan-6A (2)
QGFE.yan-6A (1)
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QSKW.mci-6A (1)
QTKW.yan

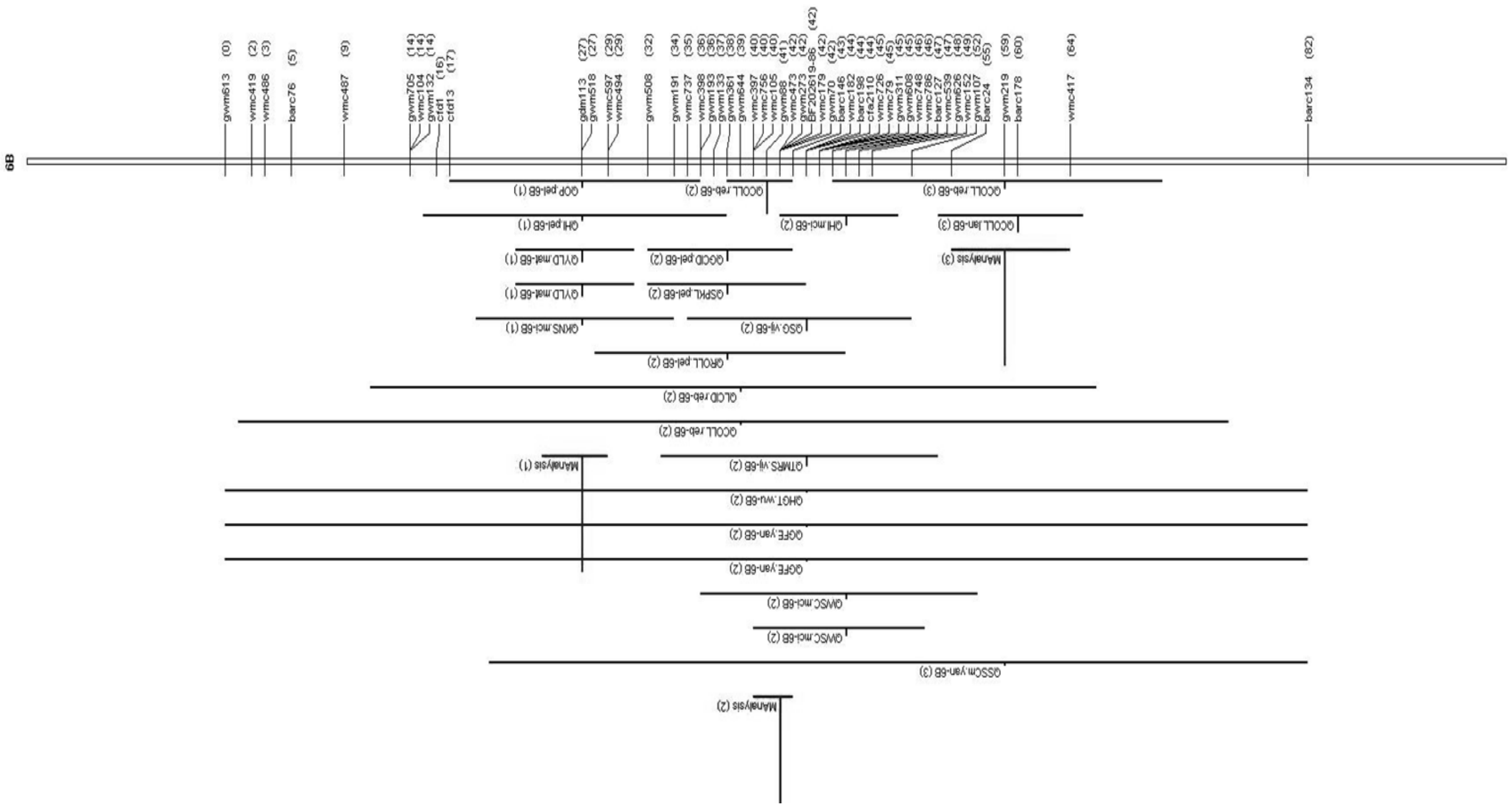


Fig. 6. Continued

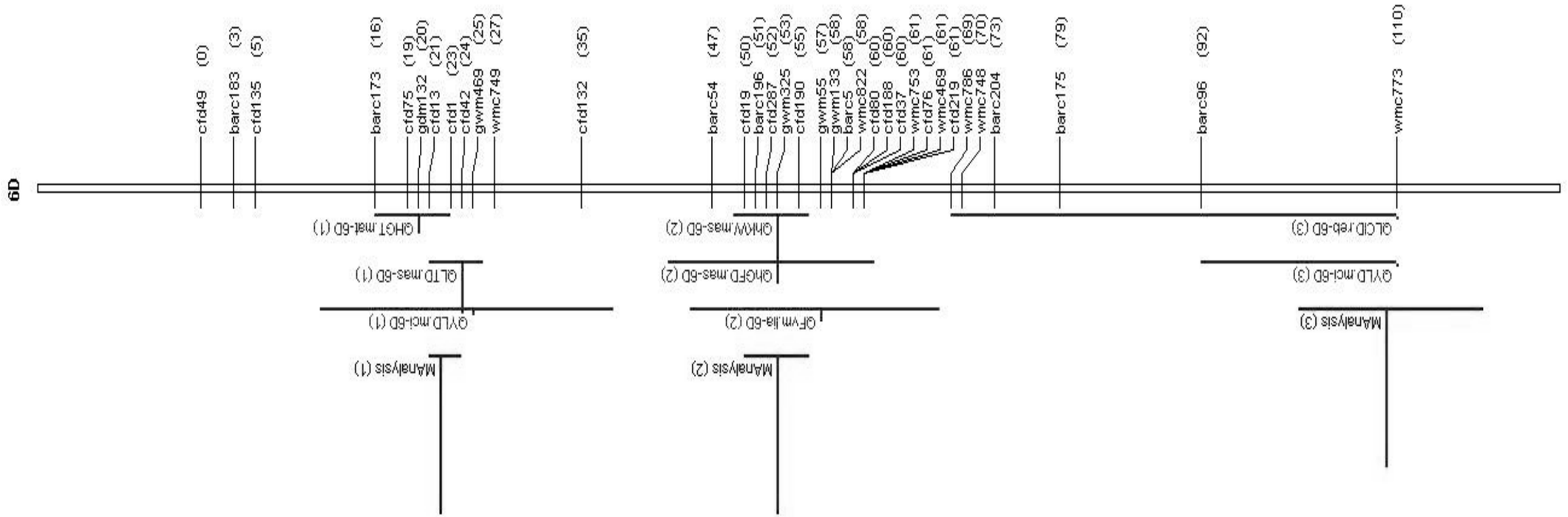
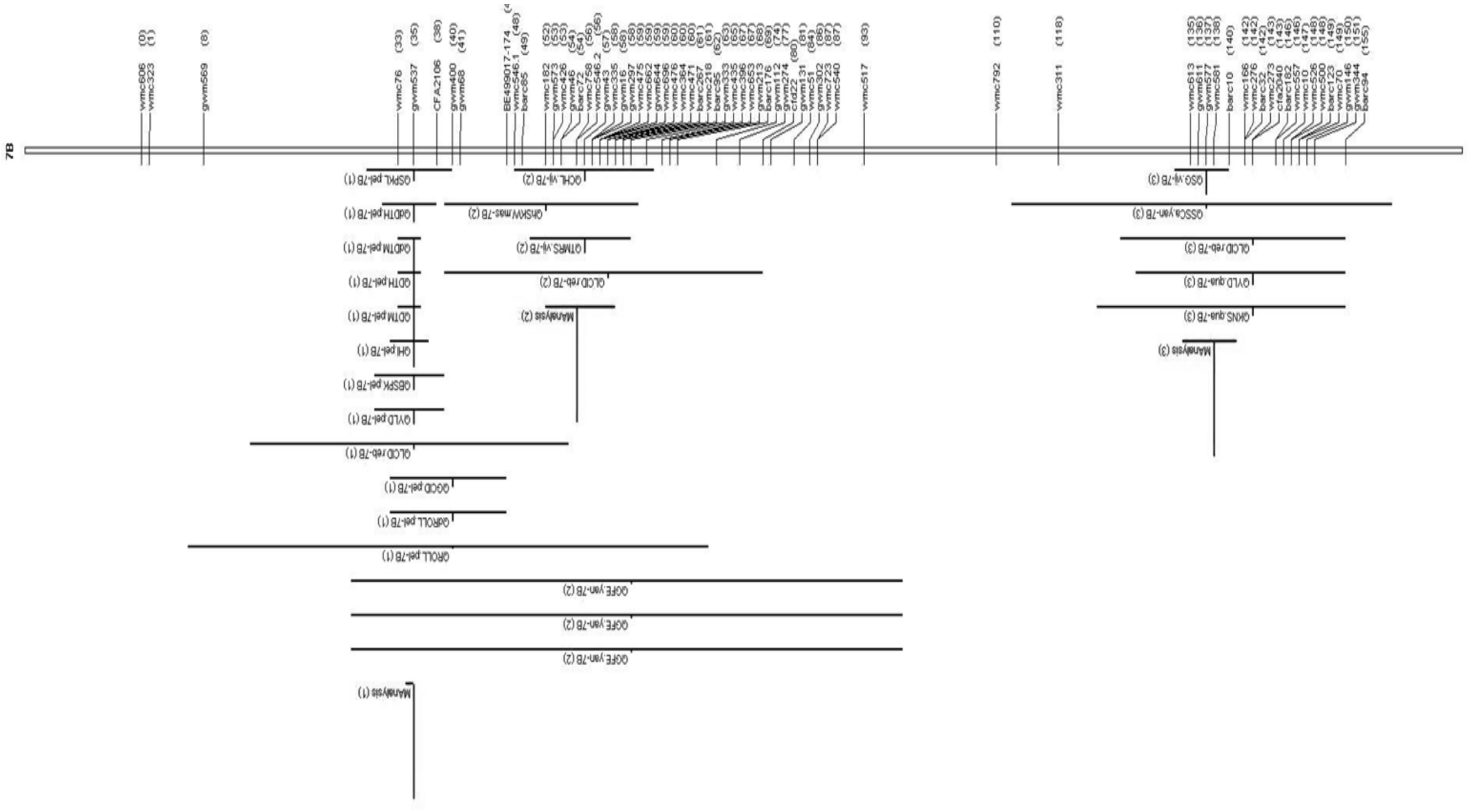


Fig. 6. Continued

Fig. 6. Continued



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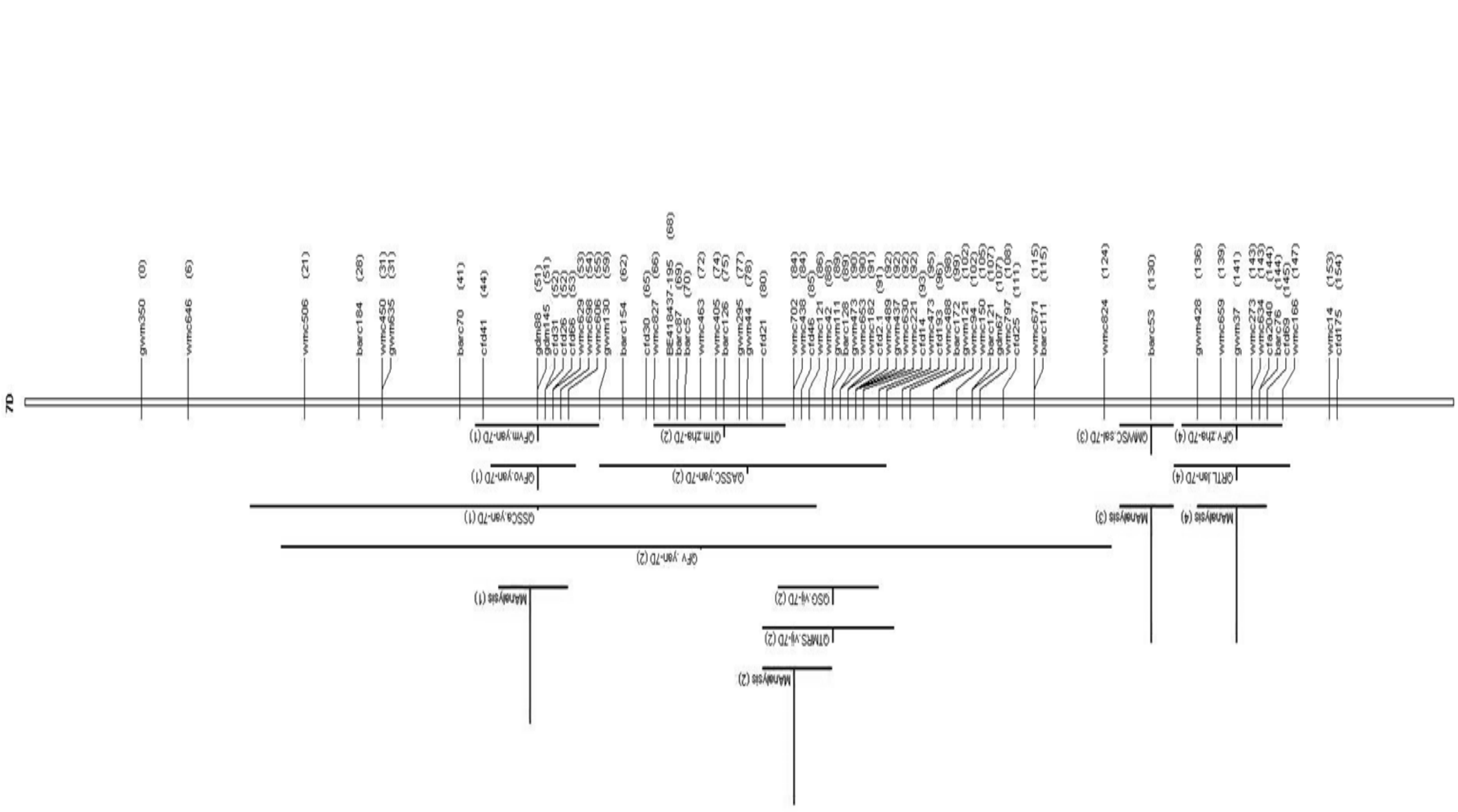

Fig. 6. Continued

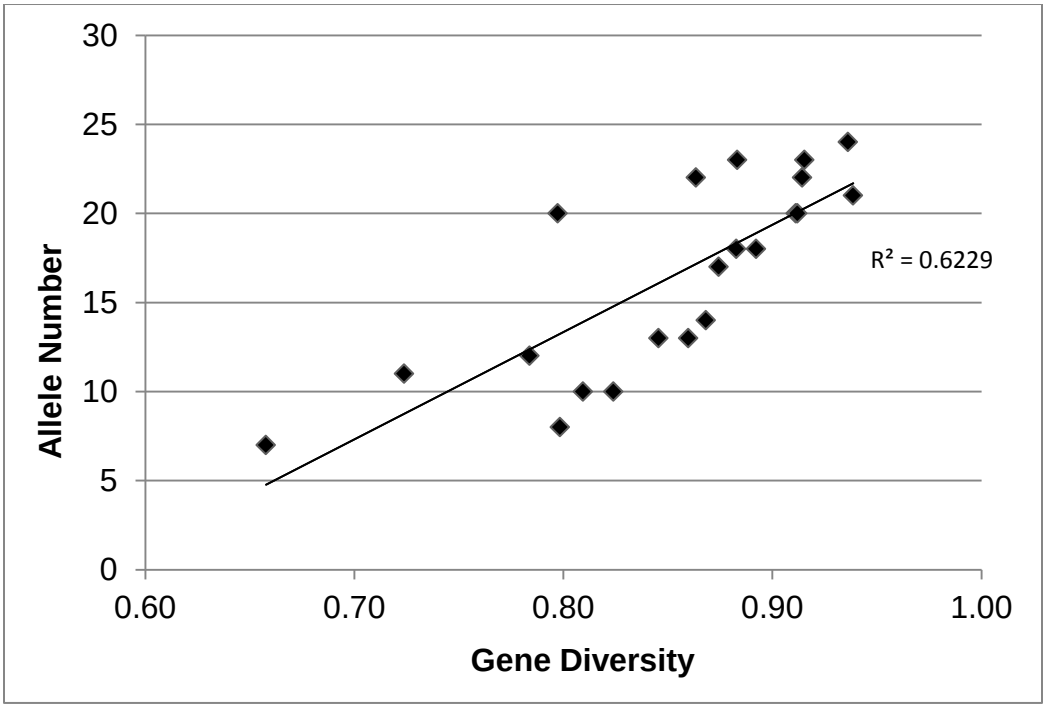

Fig. 7. Relationship between gene diversity and the number of allele detected in 21 SSR markers.

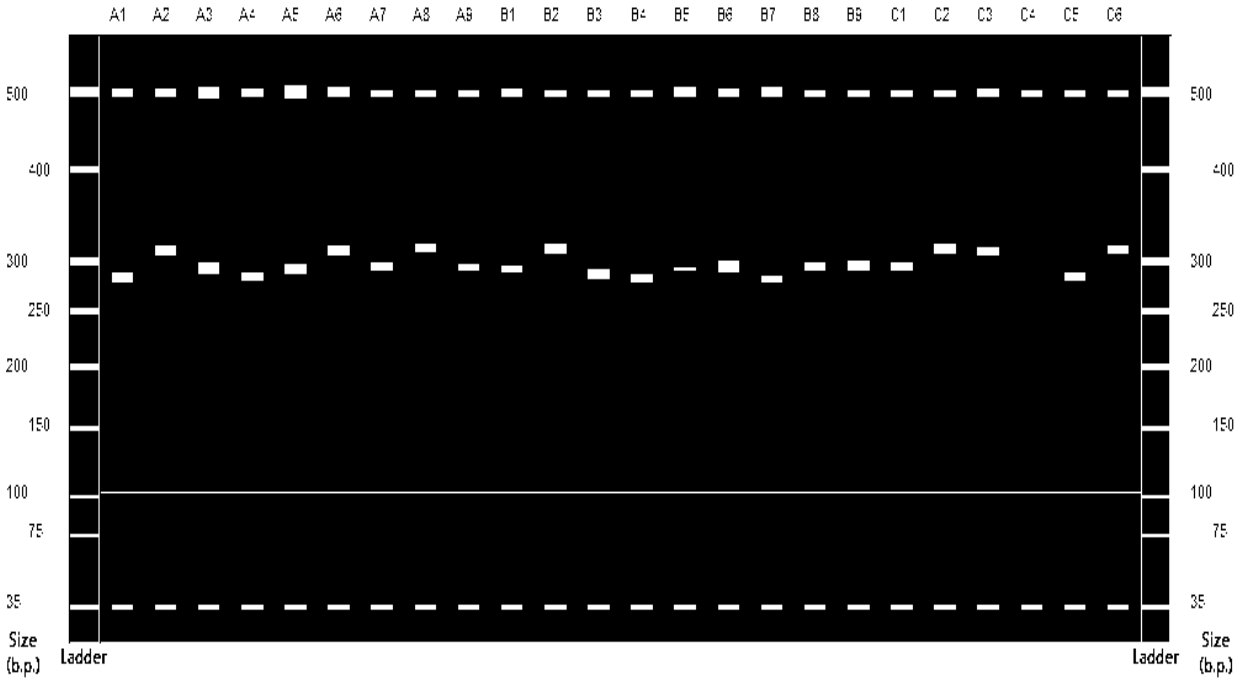

Fig. 8. Partial results of alleles for the marker *gwm156* in 24 GAWN lines.

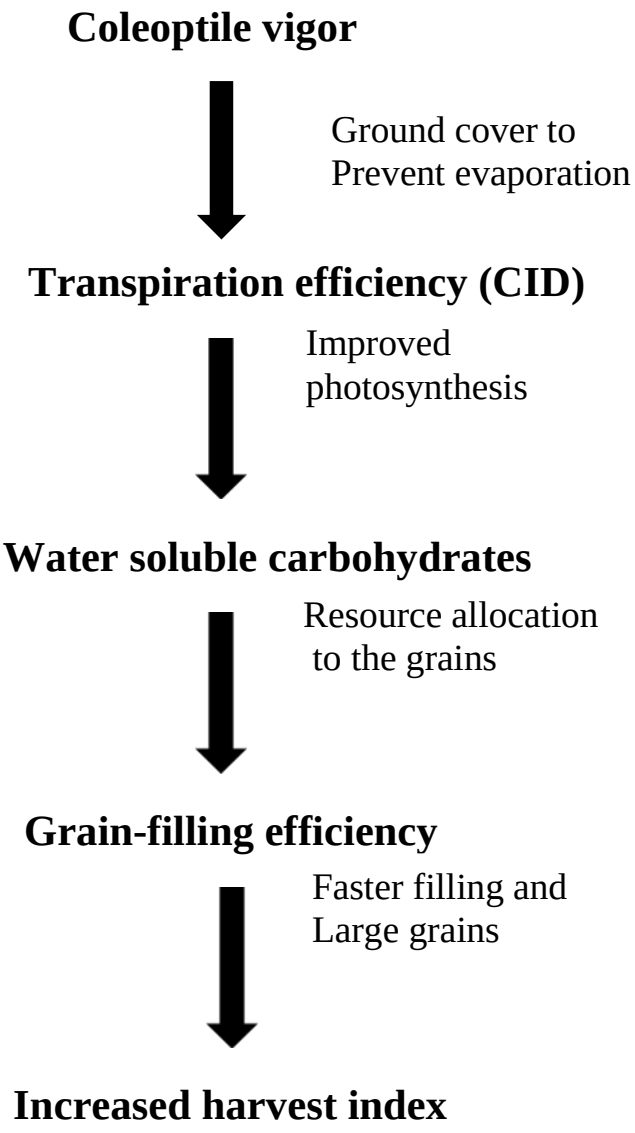

Fig. 9. Example Stress Expression Pathways for MQTL 52 (STEPS).

Chapter III

Overall Conclusions

Overall Conclusions

The QTL meta-analysis is a useful tool in order to identify stable QTL associated with heat and drought tolerance. The development of the initial database is an important and crucial step in order to minimize possible bias in the study. From the initial set of 854 individual QTL we identified 66 MTQL regions distributed across the wheat genome. From the 66 MQTL regions identified, 43 co-localized for both drought and heat stress. As well, the 95% confidence interval for the individual QTL was reduce by 16 cM in the MTQL, an important reduction that allows for a more specific target region in the development of markers for molecular breeding.

Regions on chromosomes 1A, 1B, 2A, 3B, 4A, 4B, 4D, 5A identified in this study were in agreement with previous studies. Novel QTL regions on chromosomes 1A, 3B, 3D, 6B, and 7B were also identified. In many of the sixty six regions identified, major genes were present.

Seventy four lines from the Gulf Atlantic Wheat Nursery were evaluated with twenty one SSR markers linked to important MTQL regions identified in this study. These MQTL were located on chromosomes 2B, 2D, 3B, 4A, 4D, 5A, 6B, 7A. A total of 346 alleles were detected for the 21 markers, suggesting a high level of allelic diversity present within this soft-red winter wheat germplasm.

Using traits present within MQTL regions, we develop Stress Trait Expression Pathways (STEPs) that can be used to dissect the genetic and physiological response of wheat to stress.